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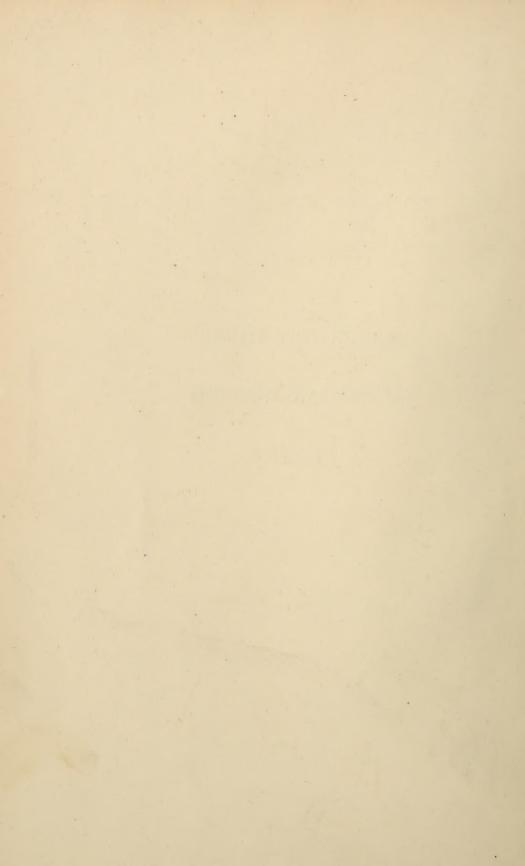
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# LABORATORY STUDIES IN MAMMALIAN ANATOMY

WILDER



# LABORATORY STUDIES MAMMALIAN ANATOMY

BY

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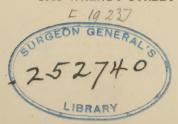
SECOND REVISED EDITION



PHILADELPHIA

P. BLAKISTON'S SON & CO.

1012 WALNUT STREET



QL 812 W673L 1923

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PRINTED IN U. S. A.
BY THE MAPLE PRESS YORK PA

MAY 14 '23 / COLA 704582

# **PREFACE**

These outlines are not intended to supply the place either of a teacher or a text-book. They are the result of several years' experience in an attempt to work out an elementary laboratory course in anatomy which might serve as a scientific basis for an accompanying course of lectures in Human Anatomy and . Physiology for undergraduates in Smith College.

Because of the impracticability of the use of human material for this work, except in the case of the skeleton and of certain demonstration dissections, the attempt has been made to base the laboratory work upon the dissection of a variety of mammals, using freely, for comparison, as large a supply of accurate manikins and models illustrating the anatomy of the human body, as it is possible to obtain. As material for laboratory dissection, therefore, the course here outlined makes use not only of the smaller mammals, but of such portions of the larger mammals as may be easily obtained in quantities sufficient for large classes through the agency of local markets or directly from abattoirs.

For the smaller mammals, rabbits, white rats, and guinea pigs are used in most cases rather than cats, because of the great ease with which these rodents may be bred upon our own premises, and the consequent relief from the vexations of spirit arising from the morbid sentimentality, and unjust criticism, which are the invariable accompaniments of an attempt to obtain a sufficiently large number of cats to supply the needs of a laboratory. There is no reason, however, why cats or dogs should not be substituted for rodents for much of the laboratory work, provided a sufficient number of specimens can be obtained, and for the most part the same outlines of work could be used with such slight modifications as the teacher would naturally suggest, or the intelligent student discover.

Considerably more is included in this outline than can be done by the average undergraduate class in 150 hours of laboratory vi PREFACE

work, *i.e.*, in a year of work with five hours of laboratory work weekly. This gives considerable flexibility to the course, since certain of the exercises may be omitted; or they may be abbreviated or given in the form of demonstrations on the part of the teacher. Moreover, an outline which covers more ground than the class as a whole can cover, gives much opportunity for optional work on the part of those students who work more rapidly than the majority, or who have more time to devote to the subject.

The course here outlined has been found to have not only a large practical value as a basis for intelligent living, but experience has shown that it serves also as an excellent introductory college course in Zoölogy, (1) since its practical personal appeal is more generally felt by the beginning student, whose knowledge of the content of general Zoölogy is often slight and whose interest in and taste for the subject is most naturally developed by beginning at the point of personal interest; (2) since the course deals not merely with adult Human Anatomy and Physiology but involves a study and comparison of many forms and stages of development, and thus gives a basis for understanding Comparative Anatomy, Histology, Comparative Physiology, and Embryology; (3) since the course gives training in all the usual methods of laboratory study, such as the study of dissections, macroscopic and microscopic study and interpretation of sections in various planes, drawing conclusions from sets of measurements. and the performance of a few simple experiments involving the use of precise apparatus; and (4) since the course introduces the more usual forms of zoölogical technic, such as the preservation of material both for dissection and for microscopic study, the injection of circulatory systems, the use of the microscope, and the preparation of microscope slides by teasing, by smears, and by sectioning.

It is because of the nature of the course as a possible introduction to other work in Zoölogy that the BNA nomenclature, which has in general been followed in these outlines, has been departed from somewhat in the descriptive terms of orientation, DORSAL, VENTRAL, ANTERIOR, and POSTERIOR, which I have endeavored to use consistently in their comparative morpholog-

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ical sense. It will be noted, however, that no attempt is made here to distinguish technical terms and technical names by the use of a different type, since it is believed that a rational nomenclature is nothing more than the most exact form of scientific language, and should be learned and used by the student as a language, rather than as a set of terms and names. Following this idea it is frequently found advisable to introduce the name of a structure or feature without information as to its exact location, when that name is such that the thoughtful consideration of its meaning on the part of the student will suffice to show its application; and it is my opinion that every effort should be made to induce the student to rely upon his own knowledge of Latin and Greek, as well as English, to help him in understanding and applying new terms.

In many parts, the outline consists rather of a memorandum of the structures and relationships to be studied than of a set of definite directions for work, to be blindly and implicitly followed. This is the intentional expression of the feeling that, in the matter of dissection and study, the sooner the student learns to rely upon his own judgment and ingenuity the better, and that, under proper restrictions, individuality in the method of working should be developed even at the occasional expense of time and material.

With regard to the method of recording the work done in the laboratory, it cannot be too strongly or too frequently emphasized that such records, whether in the form of notes or drawings, are wholly for the student's own personal use, and that with him lies the whole responsibility for their accuracy. They can have no value except as an expression of what the student has learned from his own study of laboratory material, and they should be as individual in their form as are the students themselves in their methods of study. Laboratory drawings, to express the true scientific attitude, should be in the nature of working drawings rather than of finished productions, and should be left in a sufficiently plastic form to admit of modifications and additions, if at any time a more accurate or complete knowledge of the object is obtained. Thus, while drawings should be neatly executed and labeled, for a student to labor for

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finished artistic effect, is not only inadvisable from the standpoint of time, but often leads to a sacrifice of scientific accuracy.

In the preparation of the second edition of the book a considerable rearrangement of the subject matter has been made because in the actual use of the book at Smith College the various instructors have found that such a rearrangement constituted a more logical development of the subject as well as a more convenient method of administering the course. For these suggestions and many others which have been embodied in the revision I wish here to express my gratitude to the former and present members of the Department of Zoölogy of Smith College who have been my colleagues in the teaching of this course.

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# MAMMALIAN ANATOMY

### I. GENERAL ANATOMY OF THE MAMMALIAN BODY

### A. THE BONY FRAMEWORK.

Material.—Mounted skeletons of Man and other mammals, such as the orang, gibbon, cat, rabbit; disarticulated human skeletons; also manikins.<sup>1</sup>

Methods.—Identify the structures mentioned in the outline, by the aid of any good text or reference book on human or mammalian anatomy. Note the differences in relationships involved in man's assumption of an erect position.

The aim of this general study of the skeleton should be (1) to become familiar with the bones and the groups of bones in their relation to each other and to the external form of the body, and (2) to be able to identify separate bones at sight to the extent of knowing the names of the larger and more distinctive ones, and the name of the group to which the smaller ones belong. Separate bones should always be identified and distinguished as right or left in the case of paired bones, referring them to the articulated skeleton and thus learning to think of them in their correct position in relation to the body as a whole.

Terms of Orientation.—To orient any structure of the body is to place it in its correct relationship to the body as a whole, *i.e.*, in the relation which it had when in place in the body. In the quadruped animal in the normal position, with the axis of the body horizontal, the surface or region of any part of the body which is directed upward is described as dorsal in position, while that which is directed downward is ventral; the surface or region

<sup>1</sup> In addition to the usual types of anatomical manikin, the manikin of B. Suzuki, especially adapted to the study and location of superficial features, will be found useful. This manikin, which represents a Japanese athlete with all external features slightly exaggerated, has a washable surface, neutral in color, and may thus be used with crayons of any color for demonstration purposes or for class drill in the location and identification of superficial parts. See *Anatomischer Anzeiger*, Vol. 37, Sept. 17, 1010.

which is directed forward is anterior, that directed backward is posterior; the surface or region which is directed toward the median vertical plane is medial, that directed away from this plane is lateral; in the free limbs, proximal applies to a region nearer to the trunk and distal to a region farther away from the These terms are also used to describe the relative location of different parts, one part being anterior to, dorsal to, or medial to, another. In the free limbs, the surface toward which the bending of the limb as a whole takes place (i.e., at the elbow or knee), is the flexor surface, while the opposite surface is the extensor surface. In the case of a body like the human body which has come to take an erect position, the terms of orientation may be applied as if the body were still in the horizontal position.<sup>1</sup>

## 1. Grouping of Parts.

Record your study by a conventionalized diagram of the human skeleton on as large a scale as the size of the page of your note book affords<sup>2</sup> showing relationships and names of the groups of bones. and the numbers of the bones of each group, with the names of individual bones so far as given in the outline. In this case, as in all laboratory work, supplement the diagram freely with notes explaining points which the diagram does not show.

# (a) Axial Skeleton.

**Skull.**—Cranial region, facial region. Compare man with other mammals as to the relationship of these to each other in relative size and location.

Vertebral Column.—Note number of vertebræ in each group.

Cervical vertebræ.

Thoracic vertebræ (rib-bearing).

Lumbar vertebræ.

Sacrum (sacral vertebræ).

Coccyx (caudal vertebræ).

<sup>1</sup> The BNA (Basle Anatomical Nomenclature) which is applied to the human body without reference to its comparison with other forms, uses posterior and anterior as synonymous with dorsal and ventral respectively, and substitutes superior and inferior for anterior and posterior respectively.

<sup>2</sup> A convenient size for laboratory note books for this course has been found to be about 10 X 14 inches. A good quality of unruled, smooth paper, of rather light

weight, is recommended.

**Ribs.**—True, false, floating—note number of each and possibilities of variation.

Sternum.—Note relation to the ribs.

# (b) Appendicular Skeleton.

### Girdles.

Pectoral.—Clavicles, scapulæ.

Pelvic.—Ossa coxæ, each consisting of iliac region, ischial region, and pubic region.

# Appendages.

Anterior (Arm).—Humerus, radius and ulna, carpus, metacarpus, phalanges.

Posterior (Leg).—Femur, patella, tibia and fibula, tarsus, metatarsus, phalanges.

# 2. Cavities Outlined by the Skeleton.

**In Head.**—Cranial region of the neural cavity, auditory cavities, orbital cavities, nasal cavities, mouth cavity.

In Trunk.—Spinal region of neural cavity, or spinal canal (continuation posteriorly of the cranial cavity); body cavity (or cœlom), divisible into thoracic, abdominal, and pelvic regions.

# 3. Identification of Superficial Bony Parts upon the Living Body.

Method.—By palpation locate upon your own body, or that of a fellow-student or a laboratory subject, those bony surfaces, ridges, and other protuberances which are superficial and thus readily felt through the skin. By comparison with an articulated human skeleton, and with anatomical manikins, discover the identity of each superficial region thus located, and from the following list, with the aid of anatomical atlases and reference books, learn the names of these superficial bony parts. Record the location of the superficial bony parts thus identified, upon outline drawings of the human body, ventral, lateral and dorsal views, supplied by the laboratory.

In Head.—Dome of cranium, from the occipital protuberance to the superciliary arches; mastoid processes, zygomatic arches,

<sup>&</sup>lt;sup>1</sup> Outline drawings of the human body in ventral, lateral, and dorsal aspects may be readily traced from various art atlases by the individual students, or such a tracing may be manifolded by means of a mimeograph, hectograph, or other manifolding apparatus.

malar surfaces, nasal bones, mental protuberance, angles of jaw; within the mouth, alveolar ridges, hard palate, coronoid processes of lower jaw.

In Trunk.—Spinous processes of all the vertebræ, especially the 7th cervical (vertebra prominens), end of coccyx; anterior margin of the manubrium of the sternum (the jugular notch), posterior end of sternum (the xiphoid process), outline of costal cartilages from the 7th to the 10th (forming the superficial boundary between thorax and abdomen), outline of ribs (nipple between the 4th and 5th).

In Pectoral Region and Arm.—Medial half of clavicle, acromion process of scapula, coracoid process of scapula, spine of scapula, vertebral border of scapula; larger tubercle of the humerus; lateral and medial epicondyles of the humerus; olecranon of ulna; ridge of shaft of ulna, styloid process of ulna; head of radius; distal fourth of shaft of radius; styloid process of radius; the pisiform bone, the distal ends of metacarpals; proximal and distal ends of the rows of phalanges.

In Pelvic Region and Leg.—Crest of ilium, sciatic tuber, pubic arch, pubic symphysis, greater trochanter of femur, lateral and medial epicondyles of femur, patella, lateral and medial condyles of the tibia, tuberosity of tibia, crest and medial surface of tibia, medial malleolus, lateral malleolus; tuber of the calcaneus (heel), tuberosity of 5th metatarsal, proximal and distal ends of rows of metatarsals and phalanges.

# B. GENERAL RELATIONSHIPS AND DISTRIBUTION OF MUSCLES.

# Demonstration of Muscle Relationships and Distribution in a Freshly Killed Mammal.<sup>1</sup>

Note the readiness with which superficial bony parts may be located through the skin by palpation. Note that in skinning

<sup>1</sup> The specimens used in this study of the general distribution of muscles (preferably cats or rabbits) may be very conveniently preserved for the later detailed study of muscles by the method given on p. 52. If, on the other hand, the supply of material is insufficient to allow another set of fresh specimens for the study of the viscera, immediately following, the study of the general distribution of muscles may be made first from manikins and casts, and the demonstration of the actual muscles can then be taken briefly when the freshly killed specimens for the study of the viscera are skinned.

and dissecting an animal, it is important to use well-sharpened scalpels, which should be kept in good condition by frequent sharpening during the dissection. During the process of dissection the preparation must be kept from drying by frequent applications of water. During the removal of the skin note its thickness, and the thin layer of subcutaneous muscle or panniculus carnosus, with its lines and regions of deeper attachment. Observe, incidentally, the loose connective tissue, or areolar tissue, which holds all parts together and is particularly well seen in the subcutaneous region where it holds the skin loosely to the underlying muscles. Note fibrous connective tissue membranes (aponeuroses and fasciæ) covering certain muscle masses and serving for the attachment of some of the muscles. Numerous blood vessels, both veins and arteries, and many nerves, will be seen during the process of skinning.

In the skinned specimen, compare the general proportions of the body with those of man, and note in the latter the modifications of structure and proportions due to the erect attitude.

Note that the musculature is so applied to the bony skeleton as to fill in its hollows and spaces, and leave in general only the more prominent bony processes and surfaces uncovered and hence superficial in location. (For names of these cf. A<sub>3</sub>, pp. 3 and 4.)

Important muscle masses in different regions of the body, with names of those muscles which are superficially located and thus form prominent external contours of the body:

Facial or Mimetic Muscles.—Note that these include the muscles of the external ears.

Muscles of the Lower Jaw or Mandible.—Note particularly the temporalis and masseter muscles.

Muscles of the Neck.—(The sternocleidomastoideus so prominent in man, is here represented by two inconspicuous muscles.)

Muscles of the Shoulder and Chest.—Note particularly on the dorsal side the trapezius muscle and the latissimus dorsi; on the central side the pectoralis muscle; the boundaries of the axilla formed by the edges of the latissimus dorsi and the pectoralis (dorsal and ventral "tendons" of the axilla); the serratus anterior, appearing on the lateral thoracic wall in the space between the

pectoralis and the latissimus dorsi; the deltoid muscles covering the shoulder joint.

Muscles of the Vertebral Column.—Note that it is only in the lumbar region that these are not covered by superficial muscles of other groups, and thus contribute to the external contours of the body.

**Intercostal Muscles.**—Note that these muscles fill in the spaces between the successive ribs.

**Abdominal Muscles.**—Note particularly the two rectus abdominis muscles, arranged longitudinally one upon each side of the midventrally located linea alba, which extends from the posterior end of the sternum to the symphysis pubis. The other abdominal muscles are disposed in three layers forming the lateral and latero-ventral walls of the cavity.

Muscles of the Hip Region.—Note particularly the gluteus group and compare with man, in whom these muscles are so much more prominently developed to hold the legs in a straight line with the axis of the trunk.

Flexors and Extensors of the Appendages.—Among these, note, in the anterior appendage, the biceps brachii and the triceps brachii, and the flexors and extensors of the carpus and fore foot (hand), with their respective tendons of insertion. In the posterior limb, note the extensor quadriceps femoris with its relation to the patella, and the flexors of the leg, forming at the knee joint the "outer and inner hamstrings;" the triceps suræ (calf of the leg) and its tendon of Achilles, and the tibialis anterior and other extensors of the foot with their tendons of insertion. (The foot is habitually over extended, *i.e.*, "flexed" in the wrong direction.)

# 2. Identification of Superficial Muscles and Their Tendons upon the Living Human Body.

Locate these by palpation with the muscles in question strongly contracted, and identify the muscles or muscle groups by referring to manikins, plaster casts showing muscle dissections, and atlases. Record the location of these muscles and tendons upon the outline drawings of the human body, noting their relationship to superficial skeletal features already recorded.

Temporalis.

Masseter.

Sternocleidomastoideus.

Pectoralis group and ventral tendon of axilla.

Trapezius.

Serratus anterior.

Latissimus dorsi and dorsal tendon of axilla.

Deltoid.

Biceps brachii, and its distal tendon.

Triceps brachii.

Flexor group of forearm, and flexor tendons of hand.

Rectus abdominis.

Gluteus group.

Extensor quadriceps femoris (three superficial parts).

Flexors of the leg; and their tendons of insertion (outer and inner "hamstrings").

Triceps suræ and tendon of Achilles.

Tibialis anterior and its tendon.

Extensor tendons of the foot.

# C. THE CAVITIES OF THE BODY AND THEIR CONTENTS, THE "VISCERA."

**Material.**—Freshly killed specimens<sup>1</sup> (guinea pigs, rabbits, white rats or cats) Cf. with anatomical manikins of the human body.<sup>2</sup>

General Directions for Making Scientific Drawings.—All drawings should be oriented upon the page either with the anterior or the dorsal region of the part drawn uppermost; they should be made with careful attention to proportions and upon a sufficiently large scale to show clearly all of the structures studied. Use a well-sharpened hard (2H or 4H) pencil and draw with light trial lines which may be readily erased and changed as the work progresses; let every line which is finally left express a definite idea, and finish the drawing in pencil in clear-cut, definite, but never heavy outlines. Ink is inadvisable, and shading is in general ineffective and is for beginners, at least, a waste of time.

<sup>1</sup> If rodents are used, they should not be fed for several hours previous to chloroforming. The specimens used for this study of the viscera may be kept in good condition in a considerable volume of running cold water for <sup>24</sup> hours. Subsequently they may be preserved for further study in <sup>5</sup>% formalin. Because of the irritating properties of formalin, however, material preserved in this way should be thoroughly washed out in running water for several hours before it is used.

<sup>2</sup> A very convenient model for comparison of this dissection with the human body is found to be that of the torso in the position for dissection, showing the thoracic and abdominal cavities and their contents.

The drawing as a whole should be accurately labeled and dated; and each part drawn should be labeled, if only tentatively, as soon as drawn, the temporary labels to be replaced by permanent ones in the final finishing of the drawing. Usually the most convenient method of permanent labeling is that of a set of neatly dotted, ruled, leading lines, which places the names of the parts at one side where they can be neatly written or printed.

Order of Procedure in Dissecting.—Use strong forceps, scissors, and scalpels, the latter kept in a well sharpened condition during the dissection by frequent honing. Moisten the preparation frequently, or in case a very small mammal is used, dissect under water.

Remove the skin from the entire body, except from the feet and from the regions immediately surrounding the ears, eyes, nose, mouth, and the anal and urogenital orifices.

# 1. The Coelomic Cavity, and Its Contents in situ.

After a brief review of the superficial bony landmarks and general distribution of the muscles, open the abdominal cavity by a midventral incision from the posterior end of the sternum to the symphysis pubis, and make a transverse incision through the wall upon each side of the midline, sufficiently extensive to allow the ventral abdominal wall to be turned back in four flaps to expose the abdominal viscera *in situ*.

Note that the anterior boundary of the cavity is formed by the dome-shaped muscular partition, the diaphragm, which in the mammal subdivides the cœlomic cavity transversely into the thoracic and the abdominal cavities. Posterior to the level of the pubic arch the cœlom narrows and becomes the pelvic cavity.

Note the smooth, moist serous membrane, known in the abdominal cavity as the peritoneum, the parietal portion of which lines the cavity throughout while the visceral portion, as will be seen more clearly later, invests and supports the contained organs.

Identify the viscera so far as you are able without disarranging them. Most conspicuous of all is the voluminous coiled intestine. Anterior to this intestinal mass and partly hidden in the concavity of the diaphragm, are the stomach and the liver, the latter lying more on the right side, while the spleen which is located to the left of the stomach, may often be seen.

Posterior to the intestinal mass, at the level of the pubic arch, may be seen the urinary bladder, especially conspicuous if it happens to be distended with urine. If the animal is a male, certain of the accessory reproductive organs may be seen as slender pointed sacs upon either side in the posterior part of the cavity (especially in rodents). If the animal happens to be a female in advanced pregnancy, the much enlarged portions of the gravid uterus will be pushed up conspicuously into view.<sup>1</sup>

Make an outline drawing of the ventral view of the whole specimen, showing the abdominal cavity thus laid open and the contained organs in situ, and adding to the drawing as the work progresses.

Cut the pectoralis muscles from their attachment to the sternum upon each side and reflect them laterally, thus exposing the thoracic wall.

Open the thoracic cavity by removing with the scissors a wide triangular portion of the latero-ventral wall upon each side of the midventral line leaving intact the whole line of costal cartilages which form the posterior border of the thoracic cavity, the sternum, and the diaphragm, the full extent and relations of which may now be more clearly seen. Note attached to the inner surface of the sternum, the delicate mediastinum, which forms a median partition dividing the thoracic cavity into right and left pleural cavities.

Within the space inclosed between the two layers of the mediastinum, the heart will be seen enwrapped with its own serous membrane, the pericardium. The membrane which lines each pleural cavity is the parietal pleura, which is continuous along the middorsal and midventral regions with the mediastinal pleura of its own side. The lung which lies in each pleural cavity is inclosed in the visceral pleura which is a reduplicature of the mediastinal pleura dorsal to the heart.

<sup>1</sup> In case there are embryos in the uterus, these should be examined while fresh, and their relation to the various extraembryonal parts (see p. 137) noted, after which each embryo should be carefully removed with the placenta still attached to it, and hardened in some good fixative such as that for which the formula is given on p. 22. It may subsequently be preserved to use for the study of the development of mammals (p. 137), or, if of suitable size, may be sectioned for the study of the general plan of the body (p. 15).

Remove the sternum by severing its attachment to the diaphragm and to the most anterior pair of ribs, and thus lay open ventrally the whole length and width of the thoracic cavity, the extent of which should be carefully noted. Study and identify the contained viscera *in situ*.

Note the central position of the heart and observe the large blood vessels which lead into and from it. Note that the parietal pericardial membrane forms a loose sac about the heart. Carefully lay this open and observe that the reduplicature which constitutes the visceral pericardial membrane is closely applied to the surface of the heart. Note the dark red appearance of the thin-walled anterior chambers of the heart, the auricles, due to their distension with blood, and the paler appearance of the thick-walled, muscular posterior chambers, the ventricles. In life the lungs, here seen in a collapsed condition, are distended with air and practically fill the pleural cavities. (Cf. specimen in which the lungs have been artificially inflated.)

If the heart and lungs be gently drawn to the right side, the tubular œsophagus may be seen extending lengthwise through the dorsal mediastinal space. The air passages connecting with each lung unite dorsal to the heart to form the trachea. Since this is concealed in the thoracic region by the heart and large blood vessels as well as by the thymus gland, which in most mammals (but not in the guinea pig) is located in the mediastinal space anterior to the heart, it is advisable to first locate the trachea in the neck region. This may be done by carefully separating the glandular masses of the neck and then separating the two delicate ribbon-like muscles which extend lengthwise upon either side of the median line immediately over the trachea. The trachea will be readily recognized by the presence of cartilaginous rings in its walls. Dorsal to the trachea and in direct contact with it lies the œsophagus. The trachea and œsophagus may now be traced posteriorly to the narrow anterior orifice of the thoracic cavity through which they pass in company with the large blood vessels which supply the anterior region of the body.

The trachea will be found to extend anteriorly the whole length of the neck. Its anterior end is differentiated to form the larynx. The œsophagus similarly extends the whole length of the neck.

Pry open the mouth and demonstrate, by probing, the connection of each of these passages with the pharyngeal cavity into which the mouth cavity leads posteriorly. The large jugular vein is conspicuous upon either side of the neck. Of the glandular masses, the salivary glands are the most conspicuous, although numerous lymph nodules are present. There are three pairs of salivary glands each gland connected by a slender duct with the mouth cavity. The thyroid (thyreoid) glands are in most forms closely applied to the lateral surface of the larynx and trachea.

Add to the drawing already begun the ventral view of the thoracic and the neck viscera in situ.

# 2. The Neural Cavity and Its Contents in situ.

Place the specimen with the dorsal surface up and remove the muscle masses from the lateral and posterior surfaces of the skull and from the dorsal surface of the vertebral column. With bone forceps and strong scissors carefully remove the dome of the cranium bit by bit, and the dorsal portions of the consecutive vertebrae and thus lay open the neural cavity. In the cranial region observe the brain covered by the meninges which are continuous throughout the whole neural cavity. The meninges may be removed to expose the dorsal surface of the brain. From this aspect the two cerebral hemispheres, the cerebellum and the posterior part of the medulla may be seen, the latter continuous posteriorly with the spinal cord which lies in the tubular spinal or vertebral region of the neural cavity.

Draw a dorsal view of the animal showing the brain and spinal cord in situ.

3. The Digestive System. (For comparison use demonstration preparations of other mammals and human manikins and models.)

**Preliminary Dissection.**—To expose the anterior region of the digestive tract, the trachea, heart, and lungs must be removed. This is most conveniently done by lifting up the anterior region

<sup>1</sup> The dissection of the neural cavity should be made upon the first or second day, before the brain and spinal cord have begun to soften, or, if the specimen is to be preserved in formalin or alcohol, the cavity should be opened enough to permit the entrance of the preserving fluid. If the time is too short to admit of this dissection, it may be given by the teacher upon a fresh specimen as a demonstration.

of the trachea from its contact with the œsophagus and severing the trachea immediately posterior to the larynx. By using the cut end of the trachea as a stem and cutting the larger blood vessels from their connection with the heart, the whole set of organs to be removed may be detached from the membranes which hold them in place and lifted out as one mass. The œsophagus is thus exposed throughout its entire course, and may be seen to pass through the diaphragm and lead into the stomach.

Turn the whole intestinal mass to one side and find the posterior region of the digestive tract which continues as the rectum through the dorsal region of the pelvic cavity to the anal orifice. Note that in this region, where the intestine takes a straight course, one sees clearly the typical relationship of the parietal and visceral portions of the peritoneum continuous with each other through the double layers of the mesentery. In the greatly elongated and convoluted region of the intestine the mesentery becomes correspondingly elongated along the edge where it joins the visceral peritoneum of the intestine so that it is like a very full ruffle bearing the intestine along its edge. Note the extensive branching of blood vessels which lie between its two layers. In regions where different portions of the mesentery come permanently in contact extensive adhesions occur.

Beginning with the rectum trace the whole course of the intestinal tract anteriorly (opposite to the direction of motion of its contents) freeing it little by little from its attachment to the mesentery until the vicinity of the stomach is reached. Leave the loop of the intestine into which the stomach leads, intact in connection with its mesentery.

The parts of the digestive system may now be studied consecutively from anterior to posterior end. Record by drawing.

(The mouth, pharynx, and salivary glands will be studied in greater detail with the sheep's head and other material later in the course.)

Note the extent of the œsophagus, the muscular character of its walls, and its collapsed condition when empty.

Note that the stomach is a pouch-like expansion, the development of which has involved a rotation into a transverse position so that the mesentery of the stomach has formed a sac-like structure which hangs posteriorly from the greater curvature of the stomach and is known as the great omentum. (Particularly well shown in the cat.) The spleen, which is not a part of the digestive system, is inclosed between the layers of the left hand portion of this structure; and the pancreas, an important digestive gland, which lies mainly between the layers of that portion of the mesentery which supports the first loop of the intestine, also pushes itself into the adjacent region of the omentum. Ventrally the stomach is suspended by a ventral mesentery, between the two layers of which the voluminous liver is located, so that one portion of the mesentery stretches between the stomach and the liver and is known as the lesser omentum, while the remaining portion stretches from the liver to the diaphragm and forms the falciform ligament.

Look for a gall bladder (absent in some species) partly imbedded in the liver, and for a duct leading from this, or from the liver directly, to the first loop of the intestine into which it opens in close proximity to the opening of the pancreatic duct.

Note the enormous length of the small intestine and compare it with the total length of body. The first loop, into which the stomach leads and the ducts from the liver and pancreas open, is known as the duodenum. Trace the small intestine posteriorly to the place where it opens into the side of the large intestine or colon.

Note that the colon has a blind extension, the cæcum, which is very wide and voluminous in the rodents (cf. cat and man). Do you find an appendix opening into this? Compare the total length of the colon with that of the small intestine.

Remove and discard the entire abdominal portion of the digestive system leaving only a sufficient length of the rectum to serve as a landmark.

**4. The Urogenital System.** (Cf. models showing relationships in the human body.) By exchanging specimens study both sexes, and record by means of a suitable drawing of each.

The Urinary System.—Note the location of the kidneys dorsal to the parietal peritoneum and hence not actually in the abdominal cavity. Look for the suprarenal gland slightly anterior to each

kidney, but not a part of the urinary system. Remove the peritoneal covering of the kidneys and from each kidney trace the ureter which extends posteriorly, dorsal to the parietal peritoneum, as a slender duct which leads into the dorsal region of the urinary bladder near its posterior end. To demonstrate this relationship the urinary bladder must be drawn forward. Note the ventral location of the bladder, and that it lies mainly posterior to and with its anterior surface covered by, the parietal peritoneum.

Open the pelvic cavity by cutting through the pubic arch a little to one side of the symphysis, after first carefully pushing aside, in case the animal is a male, the organs which lie ventral to the arch. Lay the pelvic cavity as widely open as possible by forcibly spreading apart the two halves of the pelvic girdle, and examine the contents of the cavity from the side.

Note that in the pelvic cavity there are no serous membranes, and that the organs lie in a packing of areolar and adipose tissue. This packing should be carefully removed until the contours of the various pelvic organs can be fully seen. The most dorsal of these is the rectum, which may now be traced to the anal orifice, while the most ventral is the urethra which leads from the bladder to the external orifice in the ventral wall of the deep depression known as the urogenital sinus of the female, while in the male the urethra takes a much more extensive course through the whole length of the penis, to reach the orifice.

The Reproductive System.—In the female find the very small oval ovary upon each side posterior to the kidney and supported by a reduplicature of the peritoneum known as the mesovarium. From near the ovary a funnel-shaped orifice leads into a tiny coiled tube, the uterine tube or oviduct, which in turn leads into the anterior end of the corresponding arm of the Y-shaped uterus. All of these structures are supported by the mesovarium, which thus corresponds to the "broad ligament" of the human female. Trace the two arms of the uterus to their union posteriorly into the median portion and note that this is located dorsal to the bladder and ventral to the rectum in the pelvic cavity, and thus like these organs is posterior to the parietal peritoneum. The uterus leads into the thinner walled and more distensible tube, the vagina,

which passes posteriorly to the external orifice within the urogenital sinus.

In the male note that the testes are located ventral, i.e., external, to the pubic arch, where they lie in the scrotal sacs, a position which they have reached by descending from the abdominal cavity through the inguinal canal (cf. the condition found in some young males, before this descent has occurred). Note that in this descent the testis has carried with it (1) certain muscular elements from the abdominal wall, forming the cremasteric muscle surrounding the testis within the scrotal sac, (2) a double layer of peritoneum, the outer derived from the parietal peritoneum in the inguinal region, the inner consisting of the reduplicature known as the mesorchium, in which the testis and its associated structures lie. Note the course of the ductus deferens anteriorly through the inguinal canal forming (together with the spermatic nerve, artery and vein), the spermatic cord; its dorsal and posterior course within the abdominal and pelvic cavities to reach the dorsal surface of the urethra into which it opens; the seminal vesicle, often very voluminous, which opens into the urethra in association with the ductus deferentia; accessory glands (increasing the liquid portion of the seminal fluid), consisting mainly of the prostate gland located at the junction of the two ductus deferentia, and the bulbourethral gland lying along the dorsal urethral wall; the penis, the distal portion of which is usually withdrawn within a reduplicature of skin known as the prepuce.

Before finally discarding the specimen, remove the kidneys with a short length of the ureters attached and preserve in 5% formalin or in 70% alcohol for later study.

# D. THE PLAN OF THE BODY AS SHOWN IN TRANS-VERSE SECTIONS AND IN A MEDIAN SAGITTAL SECTION.

For the general plan of any vertebrate body, study a transverse section through the middle of the body (cœlomic region) of a dogfish, in which the relationships are less complicated than in the mammals. For camparison, study demonstration preparations made by sawing through the frozen bodies of adult mammals,

or by cutting with a sharp, thin knife the well-hardened bodies of embryos sufficiently advanced to show the typical form and arrangement of parts (e.g., guinea-pig embryos measuring from 7 to 10 cm. in length). These sections should include not only transverse sections through both the thoracic and the abdominal regions but also a median sagittal section to show the longitudinal extent and relationships of the parts studied. All these preparations should be studied under water or 70% alcohol, and, in case embryos are used, they should be studied under a dissecting microscope.

Study for the general plan of the body, especially the location and boundaries of the neural and cœlomic cavities, and the relation of the serous membranes of the latter to the walls of the cavity and to the contained organs, with the identification of the latter. Note also, especially in the transverse sections, the disposition of the muscle masses with relation to the skeleton, and compare these sections with certain familiar cuts of meat (e.g., loin and rib chops).

Draw a transverse section through the body of the dogfish, and a median sagittal section through a mammalian body, adult or embryonic. If time permits draw also a good typical transverse section through the mammalian thoracic region and a similar section through the mammalian abdominal region.

### II. INTRODUCTORY HISTOLOGICAL STUDY

For the names of the parts of the microscope and explanation of the optical principles involved in its use, the student should consult the small book which accompanies each instrument.

# Directions to be Observed in the Use of the Compound Microscope.

- r. Lift the microscope stand by handle arm, or by base. Remove dust. If necessary, clean the oculars and objectives, using for the purpose only pure tissue paper or soft clean linen, applied with a rotary motion. Always take the greatest of care to keep the microscope lenses clean, and free from contact with any object.
- 2. See that all parts of the microscope stand are properly and firmly adjusted, and that the nosepiece, and the coarse and fine adjustments by means of which the microscope tube is raised and lowered, are in proper working order. Place the fine adjustment at about the middle point of its range of action.
- 3. Place the microscope stand squarely upon the table with the pillar directly in front of you, taking care that the direct sunlight does not fall upon you or upon any part of the instrument. Adjust the height of your own seat so that you can look through the tube with ease.
- 4. If the ocular and objectives are not already in place, adjust them as follows: Place an ocular in the upper end of the tube, and with the coarse adjustment sufficiently raised to prevent contact of the objectives with the stage, screw each firmly into its proper place in the nosepiece, finally bringing the low power objective into line at the lower end of the tube.
- 5. Look through the tube (using one eye while the other is kept open), and turn the mirror in such a way that the best available white light is reflected through the aperture of the stage giving a white circular field of vision. Note that the light may be taken from any direction except from behind you, while the microscope remains squarely placed in front of you.

2

- 6. Place upon the stage the slide upon which the preparation to be studied has been mounted (see p.19) with this preparation itself over the center of the aperture of the stage.
- 7. Focus with the low power. Before doing this, lower the microscope tube, if necessary, by means of the coarse adjustment, until the lower end of the objective is about an eighth of an inch above the slide, watching the process carefully to see that the objective does not come in contact with the slide. Never lower the coarse adjustment while looking through the tube. Then, looking through the tube, with both eyes open, slowly raise the tube by means of the coarse adjustment until the outlines and details of the object can be sharply seen, the indication that the microscope is in focus. The focus may now be sharpened, and different levels of the object may be brought out by use of the fine adjustment. Never try to see what is not in focus.
- 8. If a certain region is to be studied with greater detail, change from the low to the high power. In case the microscope you are using is parfocal, the following method should be used:

With the low power objective in place, focus the microscope upon the object to be studied. Looking through the microscope, move the slide so as to bring into the middle of the field the detail which is to be studied under high power. Without changing the adjustments, turn the revolving nosepiece so as to bring the high-power objective into line with the microscope tube. A slight turning of the fine adjustment should now suffice to bring the object sharply into focus. In most microscopes the change from low to high power involves a slight downward focusing with the fine adjustment, but you should determine which direction is necessary with each instrument that you use by cautious experiment, while looking through the tube to see when the focus is sharp. Never use the coarse adjustment with the high power, but if the microscope gets out of focus turn back to the low power and start over again with the coarse adjustment.

9. Remember that the microscope is an aid to vision, and if used properly with both eyes open and used alternately, and with frequent resting for a moment by looking off at some distant object, the microscope will never injure the eyesight.

## Preliminary Practice in Focusing and Using the Microscope.

Examine, for this purpose, one of your own eyebrow hairs or an eyelash, mounted in a drop of water placed in the middle of a slide. In order to enclose this drop between two parallel surfaces, it should be covered with a clean cover-slip. This must be applied with care in order that air bubbles shall not be included, or the upper surface of the cover-slip itself flooded with the water. To obviate these difficulties hold the cleaned cover-slip by one edge by means of the forceps and, applying the opposite edge of the cover-slip to the slide at the edge of the drop which is to be covered, gradually lower the cover-slip upon the drop. Incidentally it is advisable to make one mount with air bubbles included and use these also as objects for study under the microscope.

Study the hair, thus mounted, under low power for its general form and proportion, recording these by means of a carefully planned drawing of the whole hair. Then select some one region, the location of which should be indicated upon the drawing of the hair as a whole, for the study of the minute details under the high power. Note that owing to the thickness of the object, the details studied must be selected with great care by focusing upon some definitely determined plane or surface. Draw the details thus studied.

For further practice a few fibers of cotton, linen, wool, and silk may be successively examined, mounted as above directed.

Draw carefully each object examined with sufficient detail to distinguish them from each other and to enable you to recognize such objects if at any time they become accidentally included in your preparations of other material.

# A. FRESH MATERIAL, SHOWING CELLS IN A CONDITION AS NEARLY AS POSSIBLE TO THAT WHEN LIVING.

Throughout the following study keep always in mind the fact that cells are three dimensional objects and that a correct idea of their forms cannot be gained from a single aspect. Look, therefore, for cells in various positions. Try to turn cells from one position to another by gently pushing the cover-slip along while you have the cells under observation in the microscope field. When a clear conception of the shape of a given variety of cell has been gained, make a model of it in plastilina before attempting to draw individual cells.

Finally record your study of each variety of cell by suitable drawings in which the form of the cell as seen from various aspects is shown. Make these drawings on a large scale so that the essential parts of the cell, nucleus and cytoplasm, may be shown with whatever structural details may be peculiar to that type of cell. If the material is such that the arrangement of the cells with relation to each other is shown, this also should be studied and recorded.

### 1. Cells from Animal Tissues.

Epithelial Cells Scraped from the Inside of the Cheek. - Mount this material in a drop of physiological, or normal, salt solution (.6% to .8% sodium chloride in water, the percentage of this salt which occurs in animal tissues). Cover with a cover-slip as above directed, and examine first under low, then under high power, shutting down the illumination as may be needed to bring out well the details. Note the colorless, transparent nature of protoplasm. The preparation may be stained on the slide under the cover-slip as follows: Place on the slide, in contact with the edge of the cover-slip, a drop of stain to be used, which must be in an aqueous medium when, as in this case, the material is in water or in an aqueous solution (methylene blue is frequently used for fresh or living material). At the opposite edge of the cover-slip lay a small fragment of filter paper with its edge in contact with the fluid in which the material is mounted, thereby drawing out this fluid and allowing the stain to enter. The excess of stain may be removed later by reversing the process. In any case supply some fluid at the edge of the cover-slip sufficiently frequently to prevent the preparation from drying. An aqueous solution of glycerine (50%) may be used to good advantage for this purpose, since it not only does not dry but also renders the preparation more transparent.

Study the unstained and stained preparations to determine the shape and arrangement of the cells, seeking for this purpose cells in small groups of not more than four or five. Note the deeply staining nucleus, the surrounding cytoplasm, and the thin and delicate cell membrane, with the numerous imprints made upon it by adjoining cells.

Liver Cells.—Use a tiny fragment taken from the freshly cut surface of perfectly fresh liver (salamander or small mammal recommended). Place this in a drop of physiological salt solution upon a clean slide and break it up as much as possible by means of mounted needles or fine pointed forceps, thereby making a "teased" preparation. Add a cover-slip and then by pressing gently upon its surface with the spread points of a pair of forceps, further dissociate and spread the material. Study the small groups of cells which will lie around the edges of the larger masses. Stain with methylene blue. Note in addition to the typical parts of the cell, a great abundance of granules many of which escape from those cells which have been torn and are thus distributed through the preparation, at first masking the cells themselves. Note form and size of the cells as compared with those from the mouth epithelium.

Red Blood Cells of Some Amphibian (Salamander or Frog).— Mount a little of the blood in a drop of physiological salt solution. Cover and study the preparation, staining it with methylene blue as in the previous cases. Note that these cells are isolated and hence do not show the impress of contact with other cells. Note the pale yellow color of the cytoplasm, and the colorless nucleus which takes the methylene blue stain readily. Determine the the shape of the cell by careful focusing with the fine adjustment, and by observing cells in different positions. The cells soon become shriveled and distorted, so that fresh mounts should be made when needed.

#### 2. Cells from Plant Tissues.

Spirogyra or Some Similar Alga Showing the Arrangement of Cells in a Single Row.—Mount in water and cover with coverslip. Note the green chlorophyl, the starch granules associated with it and stained a deep blue by application of a weak solution of iodine in an aqueous solution of potassium iodide (which at the same time stains the nucleus brown, an illustration of differential staining), the large vacuoles, and the relatively small amount of

cytoplasm in delicate strands. Note the thick cell wall of cellulose.

Epidermic Cells from an Onion Bulb, Showing the Arrangement of Cells in a Single Layer Covering a Surface.—Mount in water. Stain with methylene blue and study all parts and their relations with care.

A Thin Section of Potato Cut by Hand with a Section Knife and Illustrating the Arrangement of Cells in a Mass.—Mount in water. Note large size of cells, large amount of stored starch granules within the cell. These are brought out well by staining with iodine. They usually obscure the nucleus.

## B. HISTOLOGICAL SECTIONS, A METHOD OF PRE-PARING MATERIAL FOR MICROSCOPIC STUDY.

Remove from a recently killed animal as soon as possible after death, small pieces of the desired tissues<sup>1</sup> and prepare them for sectioning, by the following method:

Killing and Fixing.<sup>2</sup>—Place the fresh material in pieces not larger than a centimeter in the greatest dimension, in a considerable quantity of the following fixing fluid for from four to sixteen hours according to size:

Bouin's Fixative.

	PARTS
Picric acid, saturate aqueous solution	. 75
Formaline (40% formaldehyde)	. 25
Glacial acetic acid	. 5

<sup>1</sup> It is recommended that at this time each student begin the preparation of a piece of material mainly for later sectioning in connection with the study of various tissues and organs. Young mammals (kittens or rabbits for example) afford excellent material. It is also recommended that Necturus or some other amphibian be used, as the large size and primitive relationships of its cells render it excellent material for the beginning student. The following tissues and organs will prove of use in later work in the course: From Necturus, cartilage, voluntary muscle, intestine, liver; from some mammal, costal cartilage, voluntary muscle, intestine, liver, salivary gland, ovary, testis, artery, vein. It is possible also to obtain satisfactory results by making transverse sections through the trunk region of a half grown salamander larva, since these sections furnish excellent material for the review of the general plan of the vertebrate body, while at the same time affording an opportunity for the examination of a variety of tissues and organs.

<sup>2</sup> The length of time which material should be left in any given fixative, and the subsequent treatment after fixation, are matters which vary with the nature of the fixative itself. Directions concerning these matters should therefore always be carefully heeded and faithfully followed.

This, like any efficient killing and fixing reagent, preserves the cells without either shrinking or swelling the parts, and holds the various cell structures in as nearly as possible the same relationships that they possessed in life. From this fixing reagent the pieces must be transferred without undue handling to 50% alcohol¹ which should be changed after an hour or two to 70% alcohol. Several changes of 70% alcohol are desirable before the material is finally left in this or a somewhat higher grade of alcohol (80%) until further use is to be made of it.

**Decalcifying.**—In case the material contains any calcarious deposit (as, for example, would be the case with bone) it is necessary that this calcarious matter be removed. A reagent commonly used for this purpose is a saturate aqueous solution of picric acid to which a sufficient amount of nitric acid is added to make about 1%. In order to transfer the material to this solution it is necessary to gradually replace the 70% alcohol in which it is preserved, by water, and for this purpose the material is transferred successively to weaker grades of alcohol (55%, 40%, 20%) and finally to water.

As a general rule to insure the desired replacement of one fluid by another, the material should be left in each new fluid for from a half hour to an hour for each millimeter of the shortest dimension of the object. It should always be kept in mind that water and grades of alcohol below 70% tend to injure tissues by softening them and thus prolonged exposure to these fluids should always be avoided.

From water the material is placed in the decalcifying fluid where it should remain for 24 hours or longer.

It must then have the excess of picric acid removed by washing in several changes of water, after which it is passed through the successive grades of alcohol until 70% alcohol is reached.

¹ To mix any grade of alcohol from any higher grade: Take as many units of the higher grade as are equal to the percentage of the desired lower grade, and add to it enough water to make of the mixture as many units as are equal to the percentage of the higher grade. For example, to mix 70% alcohol from 95%, take 70 c.c. of the 95% alcohol and add 25 c.c. of water. 95% alcohol (i.e., commercial alcohol) is the grade from which the lower grades are usually obtained; 100% alcohol is very expensive and hence must always be used sparingly, and never used to mix with water for the lower grades of alcohol.

Dehydrating.—In order to use the material, thus hardened and preserved, for microscopic sections it must first have all the water removed from it by the successive transfer to higher grades of alcohol until 100% (absolute) alcohol is reached. Thus from 70% alcohol in which it is preserved (and may be kept indefinitely until needed), it must be transferred to 95% in which it should remain for several hours, and from this to 100% in which it should be kept for only a few hours at the longest, as this tends to harden and shrivel the tissues.

Clearing.—This process consists in replacing the alcohol with some oil (usually xylol or turpentine) which, being on the one hand miscible with absolute alcohol, will replace it, while on the other hand it is a solvent of the paraffine in which the material must later be imbedded, and may thus be readily replaced in turn by the paraffine. The material is placed in the clearing oil and allowed to remain until its transparent appearance, when subjected to a strong transmitted light, indicates that the oil has quite replaced the alcohol. In the clearing oil the material may be kept indefinitely, though a prolonged stay in this medium is sikely to render it brittle.

Imbedding.—This requires the use of a paraffine oven or other device for keeping melted paraffine at a uniform temperature slightly above the melting point of the paraffine used (54°-56° centigrade).² Place the cleared tissue in the melted paraffine and leave it for a sufficient length of time to insure the complete replacement of the clearing oil by the paraffine which will thus impregnate the material. The process requires about half an hour for each millimeter of thickness (least dimension). When the tissue is to be removed from the paraffine, have in readiness all of the articles and implements which may be needed, for the paraffine hardens quickly and the transfer must be made before the hardening begins.

<sup>1</sup> Acetone may be substituted in this and subsequent processes for 100% alcohol.

<sup>&</sup>lt;sup>2</sup> A very simple and convenient device for student use consists of a jelly tumbler which is first filled two-thirds full of melted paraffine and set aside to harden, after which it may be used at any time by melting the top to a depth of about an inch by bringing directly over it an ordinary electric light bulb. So long as the material to be imbedded rests upon the solid surface of the lower paraffine it is in no danger from overheating, and the amount of melting may be regulated by raising and lowering the electric light bulb.

Prepare a watch crystal or other small container by smearing its inner surface with glycerine. Pour into it enough melted paraffine to amply cover the piece of material which is to be imbedded. Transfer the material to this container by means of a slightly warmed section lifter or a strip of stiff paper. Before the paraffine begins to solidify, adjust the material to any desired position by means of a warmed instrument, and if more paraffine is needed it may be added. The material should not be in the least disturbed, however, after the paraffine has begun to solidify. Cool the paraffine rapidly by gently blowing across the surface and at the same time gradually lower the container into a dish of cold water, carrying it under the surface of the water as soon as the paraffine is hard enough to stand the pressure. Leave it under the water until the paraffine is thoroughly hardened.

Sectioning.—The block of paraffine in which the material is imbedded should be shaved down to a rectilinear form a little larger than the imbedded mass, and should be securely fastened by means of melted paraffine upon a cork or other device for holding it, taking care to so orient it as to obtain sections in whatever plane is desired. Adjust the block to the microtome with the edge of the knife parallel with two edges of the block in such a way that the knife will pass through the shorter dimension of the object. Cut sections of the desired thickness, about 10 or 15 micra (a micron is .001 mm.), for ordinary study.

Spreading Sections on Slide.—These sections, which must be handled with the greatest of care by means of forceps or a camel's-hair brush, should be placed upon the middle of a microscope slide, which has been previously smeared with albumen fixative, thoroughly rubbed in.

The sections, if not wrinkled, may then be fastened to the slide by gently warming the preparation, holding the slide high above the alcohol flame, until the paraffine surrounding the section shows signs of being about to melt.

If the sections are wrinkled, place a drop of water in contact with them before warming the slide. This drop will run under

<sup>&</sup>lt;sup>1</sup>Albumen fixative may be conveniently prepared by mixing thoroughly equal parts of filtered white of egg and glycerine, and adding a little sodium salicylate or a few crystals of thymol as a preservative.

and float the sections, and the subsequent warming should continue until all wrinkles disappear, more water being added from time to time if needed, and care being taken not to melt the paraffine. The excess of water may then be removed with a bit of filter paper, and the slide set aside for 24 hours to dry. Keep the identity of the slide by means of a temporary label.

Staining and Mounting Sections on Slide.—After the slide is dry, remove the paraffine from the sections by first gently warming the slide over an alcohol flame until the paraffine begins to melt, and then placing the slide for a minute or two in a staining jar of xylol, which will dissolve the paraffine. As the stain which is to be used is an aqueous solution, with which, of course, oil will not mix, the xylol should be removed by placing the slide in a staining jar of 100% alcohol for a few minutes, first draining off the excess of xylol upon a piece of filter paper.

From the 100% alcohol transfer the slide to a jar of 95% alcohol, and from this, without draining off the excess of alcohol, carry the slide in a horizontal position into a large, shallow dish of clean tap water in which it should be moved very slowly back and forth until all milkiness disappears and the water runs freely from the surface when the slide is lifted out of it.

The sections are now impregnated with water and will take an aqueous stain. Place the slide in a jar of this stain (hæmatoxylin, in this case) and leave it until a decided but not dark color has been imparted to it. From time to time take the slide out of the stain and rinse it in the water to see how deep a color has been acquired (cf. with the "sample" slide furnished by the laboratory). This process may take from one to ten minutes and must be carefully watched by the student that overstaining may not occur.

When the staining is completed, carefully transfer the slide in a horizontal position, to the dish of tap water, where it should be left a few minutes in order that the slight alkalinity which tap water usually possesses, may change the reddish tinge of the sections to a bluish purple. Drain and wipe off the excess of water from the slide, and transfer the slide successively to 95% and 100% alcohol, to dehydrate it.

Then place the slide in the jar of xylol to remove the alcohol and render the stained sections transparent. There should be no milkiness in slide or surrounding fluid at this stage.

When the sections are perfectly transparent (i.e., cleared) remove the slide from the xylol, drain and wipe off the excess of xylol, place a drop of Canada balsam on the sections and cover carefully with a perfectly clean cover-slip. Label the slide with the name of the structure sectioned and with your own name together with other data such as the direction of the section, thickness of section, stain, etc. Leave in a horizontal position until the balsam is hard, taking care never to pile slides upon each other.

# C. PERMANENT MOUNTS (DEM. SL. COLL.¹) SHOWING EXAMPLES OF CELLS FROM DIFFERENT TISSUES.

Study each preparation with care, noting in each case the source of the material, and the method of preparation which has been employed. Make drawings to show your conception of each type of cell studied with the arrangement of cells when this is shown by the preparation.

Cells of Epithelial Tissue, Showing the Characteristic Arrangement of Cells to Form a Continuous Layer.—(E.g., a surface mount of the outer layer of epidermis of a salamander or frog obtained from a recent moult.)

Cells of Skeletal Tissue, howing the Characteristic Wide Separation of the Cells by Intercellular Material.—(E.g., a section of 10–15 mm. pig embryo showing embryonal skeletal tissue with stellate cells.)

Cells of Muscle Tissue, Showing the Characteristic Elongated Form of Contractile Cells.—(.Eg., a teased preparation of smooth muscle tissue from the muscle coat of the intestine of a cat.)

Cells of Nerve Tissue, Showing the Characteristic Branched Form of Cells with Wide Separation of the Cell Bodies.—(E.g.,

<sup>&</sup>lt;sup>1</sup> The term Demonstration Slide Collection (Dem. Sl. Coll.) is used to designate such slides as may most advisably be arranged by the teacher for the students to examine, rather than given out to the students. It is recommended that such a slide be accompanied always by an adequate explanation or diagram, or by a suitable reference to some text-book of histology.

a smear preparation<sup>1</sup> from the gray nerve substance of the spinal cord of the pig.)

Reproductive Cells, not Forming a Tissue in the True Sense.—(E.g., growing ova, in place, in sections of a mammalian ovary.)

# D. PERMANENT MOUNTS SHOWING CELL DIVISION BY MITOSIS.

Study slides (Lab. Sl. Coll.)<sup>2</sup> which show the cells of some tissue in which rapid growth is in progress, where, among the ordinary "resting" cells of the tissue certain cells may be found which are in the process of mitosis. (E.g., sections through the growing tips of onion roots; or through the epidermis of salamander larvæ; or surface mounts of the thin epidermis (conjunctiva) stripped off from the front of the eye of a salamander.)

Use for comparison other preparations (Dem. Sl. Coll.) showing details of mitosis.

In each case examine first the resting cells and note the relation of cytoplasm and nucleus, and the presence of the deeply staining granules of chromatin within the latter. In the case of the dividing cells search for as many stages of the process and as many aspects of the mitotic figure as possible. Note the temporary disappearance of the nucleus as a distinct structure; the transformation of the chromatin of the nucleus into chromosomes which are ultimately to be split and distributed equally to the two daughter cells, to eventually enter into the reconstruction of the nuclei of these cells; the separation of the cytoplasm into two masses surrounding the respective nuclei. Make suitable drawings to record this study.

## E. VARIETIES OF EPITHELIAL TISSUES.

Study slides (Lab. Sl. Coll.) showing a variety of epithelial tissues. In each case try to understand first the relation of the epithelium in question to the structure of the organ of which it is a part. Determine whether the plane of the preparation is at right

<sup>&</sup>lt;sup>1</sup> For this method of making preparations for histological study, see p. 50.

<sup>&</sup>lt;sup>2</sup> By the designation Laboratory Slide Collection (Lab. Sl. Coll.) is meant a set of duplicate slides which the students use individually for study as they would do with their own preparations, had they the time and skill to make them.

angles to the free surface (i.e., a vertical section of the epithelium) or is parallel with the free surface (i.e., either a tangential section of the epithelium or a surface mount). Keep in mind the fact that in order to understand the shape and arrangement of the cells it is necessary to study them from both aspects. In case of a vertical section note the sharp delimitation of the epithelium from the connective tissue upon which it rests. Consider whether the arrangement is in one layer (simple epithelium) or more than one (stratified epithelium); what the prevailing shape of the cells is (squamous, cuboidal, or columnar); and whether there are indications of cells with special functions (protective, secretory, or motile). Record by suitable drawings on a sufficiently large scale to show the parts of the individual cells, as well as their arrangement.

The following preparations (Lab. Sl. Coll.) are suggested for the study of epithelial structures although others equally good may be added or substituted.

- 1. Sections of the gall bladder of Necturus showing a lining of simple cuboidal epithelium seen mainly in vertical section, though limited regions may be cut tangentially.
- 2. Transverse sections through the stomach and gastric diverticula of a grasshopper showing in vertical section a lining of simple columnar epithelium.
- 3. Transverse sections through the intestine of some lower vertebrate (e.g., Necturus) showing in vertical section a lining of simple columnar epithelium, with numerous secretory cells (mucous cells) among the cells of more general character; and an outer covering (visceral peritoneal layer or serosa) of simple squamous epithelium also in vertical section.
- 4. Sections through the coiled oviduct of a mammal showing a simple ciliated columnar epithelium, mainly in vertical section.
- 5. Surface mounts of the mesentery showing the simple squamous epithelium of a serous membrane.
- 6. Transverse sections of the œsophagus of a lungless salamander (e.g., Desmognathus or Eurycea) showing in vertical section a stratified columnar epithelial lining only two cells thick, with both secretory (mucous) and motile (ciliated) cells.
- 7. Vertical and tangential sections through the skin of young salamander larvæ (e.g., Desmognathus or Eurycea) showing an

epidermis composed of a stratified cuboidal epithelium only two cells thick. Note the thick cuticular border of the cells of the outer layer and the numerous distended secretory cells of the inner layer.

- 8. Vertical and tangential sections through an unpigmented region of the skin of an adult salamander (e.g., Desmognathus or Eurycea) showing an epidermis composed of a stratified epithelium of several layers, the outer squamous in form (cf. surface mounts of conjunctiva used for mitosis, p. 28) while numerous multicellular alveolar or acinous glands will be seen to lie below the general level of the epidermis, through which their slender ducts pass to reach the external surface.
- 9. Surface mounts of small pieces of the moult of an adult salamander showing the external layer of squamous cells which have thus been cast off. Look for gland pores in this.
- ro. Vertical sections through the skin of a human fetus showing an epidermis composed of stratified epithelium of many layers, the outer squamous in form. If developing sweat glands and hairs are present in the sections, note their relation to the deeper layers of epithelial cells.
- 11. Vertical section through the lining of the mouth of some mammal showing a stratified epithelium of many layers, the outer squamous in form. (Cf. study of fresh cells scraped from the inside of the human mouth, p. 20.)
- 12. Transverse section through the mammalian œsophagus showing a vertical section through its much folded squamous epithelial lining.

# III. DISSECTION OF THE FOOT OF THE PIG FOR THE MACROSCOPIC STUDY OF SKELETAL TISSUES

Material.—Fresh material obtained from the abattoir. Both anterior and posterior appendages may be used; they should be amputated well above the carpus (or tarsus). Skeletons of the appendages of pig and other ungulates (e.g., sheep, cow, horse) and of man.

Preliminary Examination.—Determine by comparison with skeletons of related forms whether specimen is posterior or anterior, and right or left. Note that the epidermis, possibly including the hoofs (nails) has been scraped off. By palpation identify the various bones and joints present. Determine the level of amputation, and study and identify so far as possible the structures shown in the cross section. Distinguish between the sections of the flexor and extensor groups of muscles (or tendons).

Compare the foot with the corresponding appendage in other ungulates, both odd and even toed, and also in man, as to (1) number of digits; (2) extent of surface in contact with the ground (ungulate type as compared with plantigrade type); (3) extent to which adjoining toes are fastened together, preventing the spread of the digits; (4) range of motion of each joint.

In making the comparison with the human foot note particularly in the ungulate foot the attempt at bilateral symmetry, the typical quadrupedal elevation of the heel process, and the total absence of anything resembling the longitudinal arch of the human foot. Which has the more primitive foot, the pig or man? In what line has each specialized and why?

# Summary of the varieties of skeletal tissues which will be met in the dissection.

## 1. Tensile or Connective Tissue.

Areolar or loose connective tissue, in many regions transformed to fatty or adipose tissue.

Dense connective tissue, wholly of the fibrous variety.

Membranous (e.g., fasciæ, sheaths of tendons, periosteum). Fascicular (e.g., tendons and ligaments).

### 2. Rigid Tissues.

Cartilage (e.g., hyaline cartilage, fibro-cartilage). Bone.

Note and record, as the dissection progresses, the physical properties of each of the above tissues.

Method of Dissection.—Remove the skin from the entire foot, being careful in the vicinity of the hoofs not to cut the underlying parts. Dissect first from the extensor side. By cutting through the sheaths of the tendons and their ligamentous loops (annular ligaments), expose the fresh glistening surface of the tendons and follow them to their ultimate insertion into the bones. Note that, in making this insertion, the tendon fibers spread out and actually enter into the formation of the sheath of the bone (the periosteum).

Similarly dissect from the flexor side, noting that here portions of the distal ends of the muscles are present; also that the tendons are much more deeply located, and that therefore their dissection involves, throughout, the removal of much more loose connective and adipose tissue, which on the flexor surface of the digits assumes the form of definite pad-like structures ("walking pads"). In the dissection of the flexor side, work out the general course of two sets of flexor tendons into each digit. Following these out in detail into one of the larger digits, note that the more superficial one eventually divides over the metacarpo-phalangeal (metatarsophalangeal) joint into two slips, which allow the deeper tendon to pass between them to its more distal insertion. Trace both the superficial and the deeper tendons to their insertion. Note in connection with the metacarpo-phalangeal (metatarso-phalangeal) joint the development of sesamoid cartilages (fibro-cartilage) and sesamoid bones.

Dissecting still more deeply upon the extensor side, study the various joint structures. Note the arrangement of the external band ligaments; the continuity of the periosteum of each bone concerned in the joint into a loose joint capsule which entirely encloses the joint; the smooth, moist synovial membrane which

lines the joint capsule; the lubricating "joint oil," or synovia, within the joint cavity; the thin layer of hyaline articular cartilage which covers the bone, thus forming the frictionless articular surface; internal band ligaments in certain of the intercarpal (intertarsal) joints.

If time permits, skeletonize your specimen, either by dissection, leaving the ligaments which hold the bones together; or by the prolonged application of steam which may be led from an ordinary steam-pipe into a tightly closed receptacle containing the foot; or by prolonged boiling. In case either of the two latter methods be used, a chemical hood is recommended. Note at various stages of the process the effect of the moist heat upon the texture and appearance of the various skeletal tissues. The skeletonized material may finally be dried and preserved indefinitely.

#### IV. HISTOLOGY OF SKELETAL TISSUES

#### A. TENSILE OR CONNECTIVE TISSUES.

#### 1. Loose Forms.

Make a preparation of **fresh areolar tissue** by spreading out on a slide by means of mounted needles, a little of the subcutaneous connective tissue or intermuscular septum from any available mammal (e.g., cat or rabbit) and when the film thus spread is dry enough to remain in place, add a drop of physiological salt solution and cover with a cover-slip. Select a thin region of the preparation. Examine first with low power, and then with high power. Note large amount of intercellular substance in the form of collagenous fibers and elastic strands. The fibers occur always in bundles and are often so fine and delicate that the high power must be used to bring out the component fibers of a bundle. The bundles usually appear in wavy lines, and frequently branch, although the individual fibers do not branch. The elastic strands are highly refractive in character, and by their abundant branching and anastomosing form a reticulum.

Irrigating the preparation under the cover-slip with a 2% solution of glacial acetic acid serves to render the elastic reticulum more conspicuous, since the acid swells the bundles of collagenous fibers and causes them to gradually disappear from view. The nuclei of the scattered cells of the tissue may also become more conspicuous under the action of the acid.

To bring the cells more clearly into view, the preparation may now be stained under the cover-slip with methylene blue, or a fresh preparation may be made and thus stained. Note the irregular form of the cells, which are mainly of the lamellar type. Can you determine any definite relationship of the cells to either of the intercellular structures?

Make drawings to show clearly your conception of each sort of intercellular structure of areolar tissue and the relation of the cells to these.

For comparison study permanent preparations (e.g., cross sections of the intestine) in which areolar tissue may be seen in place in its relation to the other tissues which make up the organ. Record by drawing.

Examine permanent preparations (Lab. Sl. Coll.) of adipose tissue (e.g., in toto mounts of thin fatty deposits in the mesentery). Note that the cells have become enormously distended and spherical in form by the accumulation of fat which has crowded the nucleus and cytoplasm to one side of the cell. Draw a few of these fat cells.

#### 2. Dense Forms.

As an example of the **fibrous fascicular variety** study permanent preparations (Lab. Sl. Coll.) of tendons. Note that in the longitudinal section the intercellular substance is seen to consist of large bundles of fibers arranged parallel to each other, with the cells flattened into the spaces between, and thus appearing in rows. Transverse sections of tendon show the cut ends of the large bundles of fibers delimited by the plate-like cytoplasmic processes of the lamellar cells which are crowded into the angles between the bundles. The septa which may be seen to separate the different portions of the tendon are of areolar tissue. Make such drawings of sections of tendon as will clearly show your conception of the structure of this variety of tensile tissue.

As an example of the **elastic variety** of tensile tissue study permanent preparations (Lab. Sl. Coll.) of an elastic ligament (e.g., the ligamentum nuchæ). In a teased preparation or in longitudinal section note that the bulk of the structure consists of a heavy reticulum of elastic strands with narrow elongated meshes. In transverse section the highly refractive appearance of the cut ends of the elastic strands will be noted, as well as the scattered cells which lie among the delicate bundles of white fibers which fill the meshes of the reticulum. Make such drawings as will show the structure of the elastic variety of tensile tissue.

#### B. RIGID TISSUES.

## 1. Cartilage.

Mount in physiological salt solution a thin transverse section of fresh hyalin cartilage (e.g., costal cartilage of some young mam-

mal). Such sections should be made by hand with a section knife, the piece of cartilage being held firmly between two pieces of pith, through which the knife passes in making the section. If several such sections are made rapidly and allowed to fall into a dish of the salt solution, among these, one will be likely to be thin enough to use.

Examine under both low and high power, selecting particularly the growing region near the periphery. Note that the rounded cartilage cells are arranged in groups, each cell and each group surrounded by a capsule, which in its outer boundary blends with the capsules of other groups, thus contributing to a hyaline intercellular matrix.

Study permanent preparations (Lab. Sl. Coll.) of stained sections of hyalin cartilage to demonstrate the above-mentioned structures. Record your observations by suitable drawings.

For comparison study and *draw* sections (Lab. Sl. Coll.) of **fibro cartilage** (e.g., intervertebral cartilage). Note that the cells are of the typical cartilage nature with hyalin capsules, but that the intercellular substance includes numerous bundles of collagenous fibers like those of connective tissue.

Similarly study and *draw* sections (Lab. Sl. Coll.) of **elastic cartilage** (*e.g.*, the cartilage of the external ear). Note that in the hyalin matrix which lies between the groups of typical cartilage cells, there is a reticulum of elastic strands with the highly refractive appearance characteristic of these.

#### 2. Bone.

Study a transverse section (Lab. Sl. Coll.) through the **dense bone** which forms the shaft of a long bone of some small mammal, decalcified and stained. Study under low power for general topography, noting the periosteum which forms its outer covering and the mass of cells of various types which fills its marrow cavity. Note that in the bony tissue itself the intercellular substance consists of systems of parallel lamellæ which follow the direction of (1) the outer surface of the bone (peripheral lamellæ); (2) the boundary of the marrow cavity (medullary lamellæ); and (3) the Haversian canals through which the blood vessels and nerves reach the various parts of the bone (Haversian systems of lamellæ); and

that there are (4) systems of lamellæ irregularly placed filling in the angles between the other systems (interstitial lamellæ). Note that the lines of bone cells indicate the boundaries of adjacent lamellæ.

Under the high power study the details of the bone cells, the flattened, nucleated bodies of which occupy crevices or lacunæ between the lamellæ, while the intricately branching cytoplasmic processes project into minute canals (canaliculi) which traverse the thickness of the lamellæ and thus make possible an actual anastomosis of the branches of neighboring cells. (Schmorl's method of staining which renders the bone cells reddish purple and the lamellæ yellow, is especially good for the study of these details.)

For comparison study under both low and high power an unstained dry transverse section through the shaft of a long bone (Lab. Sl. Coll.). Note that the method of preparation has removed all of the soft parts so that the Haversian canals, lacunæ, and canaliculi appear as empty spaces.

Examine a longitudinal section through the shaft of a long bone, unstained dry preparation (Lab. Sl. Coll.), and identify from this new point of view the structures already studied in the transverse sections.

Record the above mentioned facts by drawings (1) of a sector of a transverse section passing from the periphery of the bone to the marrow cavity, showing the various systems of lamellæ; (2) of three adjoining lamellæ with lacunæ and canaliculi as seen under the high power; and (3) of a few bone cells showing the details of their branches.

Study a longitudinal section through **developing bone**, showing the formation of bone from cartilage (Lab. Sl. Coll.). Study first with the naked eye or with a dissecting microscope and note that the section passes through the cartilaginous head of a long bone which is undergoing ossification. Draw on a large scale an outline of the whole section and fill this in with as many of the details of structure as you are able to identify in the following microscopic study.

Under low power study the various regions, beginning with the typical hyalin cartilage. Note the arrangement of the cartilage

cells in columns as the line of ossification is approached, and the disappearance of the hyalin matrix between the cells in the region of bone formation.

Note the small, deeply staining osteoblasts disposed in a layer lining the cavities thus formed, and the layers of intercellular substance which they are forming. Those osteoblasts which become included between the layers or lamellæ become the bone cells. Look for larger multinucleate cells, osteoclasts (or bone destroyers), among the cells of the marrow cavities, and applied to the surfaces of lamellæ. These, by dissolving the bone material, form the cavities of bone. Note the extent of periosteum over the surface of the bone, also portions of muscles attached to this.

#### V. THE HUMAN SKELETON

Materials.—Mounted and disarticulated human skeletons; also a variety of skeletons of other mammals, for comparison. Note that in the following study, features of bones may usually be identified by considering the meaning of the name which they bear. Reference books should be used as a help only as a last resort.

## A. THE VERTEBRAL COLUMN, RIBS AND STERNUM.

A general preliminary view of these bones and their relationships should be obtained by arranging upon the table top the entire series of vertebræ in their correct order, the thoracic ones sufficiently separated from each other to allow the pair of ribs corresponding to each to be placed in position beside it, careful attention being given to the distinction of rights and lefts. As a criterion for the arrangement of the bones, refer to a correctly mounted skeleton and apply also the test of fitting together adjacent bones by their corresponding articular surfaces. Note the gradual transition from each group of vertebræ to the adjacent group.

# 1. A Typical Thoracic Segment.

Avoiding the more modified anterior and posterior ends of the thoracic region, each student should select for study one of the more typical thoracic vertebræ (2nd to 9th inclusive) and its corresponding pair of ribs. To understand correctly the relationships of these, the vertebra next anterior to the segment selected should be included in the set of bones selected. Record the serial number of the segment.

# (a) A Thoracic Vertebra.

Orientation.—Distinguish dorsal, ventral, anterior, posterior, right and left lateral aspects.

Parts.—Body or centrum; and vertebral arch which encloses, dorsal to the body, the vertebral foramen. The arch bears a

median dorsal spinous process, two lateral transverse processes, two anterior articular processes, and two posterior articular processes.

Note in a lateral view of the two adjacent vertebræ, that the anterior vertebral notch of one and the posterior vertebral notch of the other form together the intervertebral foramen.

Articulations.—Note carefully the boundaries of articular surfaces. The body articulates with the bodies of adjoining vertebræ; the arch articulates with their arches by means of the articular processes; each transverse process bears a costal pit for the tubercle of the rib, and the body bears costal pits for the heads of the rib (tubercular and capitular articulations respectively); note that in the middle thoracic region, the capitular articulations are divided between adjoining bodies; note also the absence of tubercular articulations in the case of the more posterior thoracic vertebræ, owing to the reduction of the tubercles of the corresponding ribs.

# (b) A Pair of Ribs Corresponding to the Thoracic Vertebra Studied.

Orientation and identification as right or left.

Parts.—Body, angle, tubercle, neck, head.

Articulations.—Tubercle with transverse process of vertebra, and head with body of vertebra (or with the two bodies of adjacent vertebræ); ventrally either directly or indirectly with the sternum through the costal cartilage (except the two floating ribs).

# (c) The Sternum.

Note the division into the manubrium, body, and xiphoid process. Identify the articular surfaces for the clavicles, and for each pair of ribs.

To show the relationships of the parts of a typical thoracic segment, draw either an anterior or posterior view of a thoracic vertebra, the corresponding ribs, and a diagrammatic representation of the cross section of the sternum, all natural size, the bones slightly separated to show their complete form. Draw also a dorsal view of the vertebra, a lateral view of the vertebra, together with the adjacent anterior one, and a ventral view of the sternum, showing all the rib articulations.

# 2. Comparative Study of Other Regions of the Vertebral Column.

Study these as illustrations of regional differentiation, using the parts of the nearest thoracic segment as a basis for interpreting the vertebræ of other regions.

A Typical Cervical Vertebra (3rd to 5th).—Study as in case of the lumbar vertebra; note the presence of the foramina of the transverse processes and seek an explanation of their formation by comparison with the structure of an anterior thoracic vertebra and the ribs articulated with it. *Draw an anterior view*.

The Atlas and Epistropheus.—Study these carefully for specializations due to their function of supporting the skull; note in the atlas the absence of the body, and the consequent ring-like form of the bone, with dorsal and ventral arches, the latter forming an articulation with the tooth of the epistropheus; peculiarities in shape and position of articular surfaces (a) on the atlas for articulation with the occipital condyles of the skull, (b) on both atlas and epistropheus for articulation with each other; note the direction and extent of the motion of the head made possible by these articulations, and compare this with the range of movement of other parts of the vertebral column. Draw an anterior view of atlas, also a lateral view of both atlas and epistropheus, the bones slightly separated to show their complete shape.

A Typical Lumbar Vertebra.—Note the peculiarities in form and size of body, arch, and processes. Compare with the various thoracic vertebræ as to the slant of processes and the number and position of articular surfaces. *Draw an anterior view*.

The Sacrum.—Note the number and relation of fused component parts, and find a reason for the fusion; lateral masses formed by the fused ribs; the continuation of the vertebral canal; foramina in three series, intervertebral, dorsal, and ventral, and their relation to the component sacral vertebræ and rib elements; auricular surfaces for the attachment of the pelvic girdle. *Draw a dorsal or a ventral view*.

The Coccyx.—Note the number of component parts and the extent to which these are fused (cf. with parts of a typical vertebra and with the caudal vertebra of other mammals). Add the coccyx to the drawing of the sacrum.

#### B. THE SKULL.

### 1. Shape and General Proportions.

In this study compare as many examples of human skulls as are available and note the extent of individual variation. Some of these should be skulls which have been sawn horizontally through the walls of the cranial cavity. Determine each aspect studied with great exactness, using as the horizontal plane of reference, the plane passing through the lower margin of the orbits and the upper margin of the external acoustic meatus. This plane is known as the "Frankfort horizontal."

Lateral View.—Distinguish cranial and facial regions; determine and compare facial angles of different species of mammals and different individuals of the same species, noting degree of prognathism and human tendency in the direction of orthognathism; note extreme reduction of the jaws in certain individuals (cf. variation in this respect shown by Boston bull dogs and grayhounds); note effect of orthognathism upon the teeth as shown in the reduction of the dental arcade with crowding and atrophy of the posterior molars.

**Basal View.**—Note the position of the occipital foramen, compare with other mammals, and explain the difference.

Vertical View.—Compare relative amounts of facial and cranial regions visible in this view of the skulls of different mammals; note individual and racial variations in the proportions of the human skull ("long" and "short" heads).

Frontal View.—Note direction of axes of orbits; compare man with other mammals with regard to extent of field of vision and range of binocular vision; note correlation of small size of nasal region with large eye and brain development and with orthognathism.

Record the observations made by blocking out, in very light lines, outline drawings, natural size, of lateral, basal, vertical, and frontal views of the skull, and internal views of the floor and roof of the cranial cavity. As the study of the special features is taken up, record each of these, with care as to details of relationships, upon each view in which it is visible. Finally finish up each drawing with the usual clear cut outlines.

## 2. Special Features.

## (a) External.

Ridges, Processes, etc.—Superciliary arches, zygomatic arches, postorbital bars, temporal ridges, external occipital protuberance and crest (cf. lambdoidal ridge in those mammalian skulls in which the temporal ridges meet), alveolar ridges of upper and lower jaws, and the types of teeth which they bear (cf. human dentition with that of other mammals), pterygoid processes and hamuli, styloid processes, mastoid processes, occipital condyles; on the lower jaw note also the coronoid processes, the condyloid processes, the angles of the jaw, and the mental protuberance.

Fossæ.—Orbital, temporal, nasal, and mandibular (for articulation with lower jaw).

Foramina, Fissures, etc.—Supraorbital, infraorbital, and mental foramina, for the exit of the terminal portions of the three branches of the trigeminal nerve; mandibular foramen for the entrance into the mandibular canal of the third branch of the trigeminal nerve; piriform opening (anterior nares), choanæ (posterior nares), larger and smaller palatine foramina; lacrimal foramen; optic foramen, superior orbital fissure, inferior orbital fissure, foramen rotundum, foramen ovale, lacerated foramen, semicanal of auditory tube (Eustachian tube), carotid canal, jugular foramen, hypoglossal canal, condyloid canal, occipital foramen; external acoustic meatus, stylomastoid foramen, mastoid foramen (or foramina). All of these are paired except the occipital foramen.

# (b) Internal (within cranial cavity).

Use for this purpose a horizontally sawn skull. Note the structure of the bones of the cranium as shown by the cut edges.

In the roof of the cranial cavity note the grooves for the veins and arteries, and granular foveolæ.

In the floor of the cranial cavity note:

Ridges and Processes.—Crista galli, clinoid processes, and prominent ridges separating anterior, middle, and posterior cranial fossæ.

Fossæ.—Anterior cranial, middle cranial, posterior cranial; sella turcica; grooves for veins and arteries.

Foramina.—Numerous perforations in the cribriform plate of the ethmoid for the olfactory nerves; optic foramen for the optic nerve; superior orbital fissure for motor nerves to the eyeball muscles (oculomotor, trochlear, and abducent), and for the first branch of the trigeminal; foramen rotundum for the second branch of the trigeminal; foramen ovale for the third branch of the trigeminal; internal acoustic meatus for the facial and acoustic nerves; jugular foramen for the glossopharyngeal, vagus, and spinal accessory nerves; hypoglossal canal for the hypoglossal nerve. (Cf. demonstration preparation of the human skull showing the course of the cranial nerves.)

## 4. Bones of the Skull.

Identify, by means of disarticulated skulls, the extent and boundaries of the individual bones, and add these outlines to all of the drawings in so far as they are visible, showing particularly the relation of the individual bones to the various features of the skull. In the final finishing up of the drawings, leave the outlines of the bones in lighter lines that they may not be confounded with the features of the skull as a whole.

## (a) Cranium.

Frontal—1.

Parietals-2.

Occipital—1.

Temporals—2.

Auditory ossicles—3 pairs (Cf. demonstration preparations.)

Ethmoid—1.

Sphenoid—1 (In adult fused with occipital.)

# (b) Face.

Nasals-2.

Maxillaries-2.

Zygomatics—2.

Palatines—2.

Inferior turbinated—2.

Lacrimals—2.

Vomer-1.

Mandible—1.

Hyoid bone—r. (A complex of several bones and cartilages, in man suspended by ligaments from the styloid processes, and not present in the usual dry preparations of the skeleton. Cf. demonstration dissection of tongue and larynx.)

#### C. THE APPENDICULAR SKELETON.

Study both articulated and disarticulated skeletons of the girdles and the appendages; learn in each case to orient completely the bone in question, and to give definite reasons for your orientation in exact scientific language; work out the relation of adjoining bones to each other as indicated by the articular surfaces where they come in contact.

Draw at least one view of each bone, preferably, in the case of the free limb, the same aspect (extensor or flexor) of all the bones of the limb, so placed as to show their relation to each other but sufficiently separated to show the entire outline of each bone. In case a bone is drawn separately, include in the drawing the corresponding articular region of adjoining bones.

## 1. The Anterior Appendage.

### (a) The Pectoral Girdle.

Scapula.—Note triangular shape; costal (ventral) and dorsal surfaces; three margins, anterior, axillary, and vertebral; spine ending in the acromion with the articular surface for the clavicle; coracoid process; scapular notch; supraspinous and infraspinous fossæ; subscapular fossa; head, with glenoid cavity for articulation with the humerus; neck.

Clavicle.—Note double curve; the rounded sternal extremity and its articular surface, and the flattened acromial extremity and its articular surface; the smooth anterior (superior) surface and the roughened posterior (inferior) one; the coracoid tuberosity.

# (b) The Free Limb.

Humerus.—Head (articular surface for the scapula); anatomical neck; surgical neck; larger and smaller tubercles, with a groove for the tendon of biceps (the intertubercular sulcus) lying between; deltoid tuberosity; medial and lateral epicondyles, with ridges extending from these up the shaft; trochlea, the articular surface for the ulna; capitulum, the articular surface for the

radius; groove for the ulna nerve; coronoid fossa; olecranon fossa (this may pierce the bone and open into the coronoid fossa thus forming the supratrochlear foramen).

Ulna.—Olecranon; coronoid process; semilunar notch for the trochlea of humerus; radial notch for head of radius; interosseous crest; head (at distal end), with styloid process and articular surface for the radius.

Radius.—Head with pit (fovea) for articulation with the capitulum of the humerus; articular circumference for the radial notch of the ulna; tuberosity for the insertion of the biceps muscle; interosseous crest; distal end with styloid process, ulnar notch, and articular surfaces for the naviculare and lunatum.

**Carpus.**—Proximal row consisting of the navicular, lunate, triquetral, and pisiform bones; distal row consisting of the greater and lesser multangular, the capitate, and the hamate bones. Note how the bones of this row are associated with the separate digits.

Hand.—Note the five digits with similar bones arranged in successive ranks, the proximal rank consisting of metacarpals, the remaining ranks of phalanges. Determine the number of these in each digit. Note the characteristics by which phalanges are distinguished from metacarpals, and proximal, middle, and distal phalanges from each other. Look for indications in the form of the articulations, of greater mobility of the thumb than of the other digits.

# 2. The Posterior Appendage.

## (a) The Pelvic Girdle.

Ossa Coxæ.—Note that each of these is made up of three components which fuse into one bone, the os coxæ, at about the 13th or 14th year. The three components meet in the center of a deep cup, the acetabulum, in the formation of which they partake about equally. Note the linea terminalis which, when the two ossa coxæ and the sacrum are fitted together, forms the boundary line between the upper and lower pelvic cavities.

The **os ilium** is the broad shovel-like region which projects anteriorly and laterally and articulates with the sacrum. Note the lateral and medial surfaces; the iliac fossa of the later; the crest, and the two ventral, and two dorsal spines; posterior to the latter the greater sciatic notch, in the middle of which the ilium joins the ischium; the auricular surface on the medial surface of the bone for the articulation with the sacrum.

The **os ischii** is the most posterior component, having the form of a loop. Note the sciatic tuber, which supports the weight of the body in sitting; the sciatic spine, separating the greater from the lesser sciatic notch; the ramus, meeting the ramus of the pubis and bounding the obturator foramen.

The os pubis is the most ventrally situated component. Note the body forming, with the corresponding portion of the other side, the pubic arch; the symphyseal surface along which it comes in contact with the pubic bone of the other side, in the midventral line; the ramus, meeting the ramus of the ischium.

Sex Differences Shown by the Pelvic Girdle.—These are best seen when the two ossa coxæ and the corresponding sacrum are fitted together and held firmly in place. Compare as large a number of examples as possible as to the subpubic angle formed by the posterior margins of the pubic rami, narrow (averaging 58°) in the male, wide (averaging 76°) in the female; shape of the iliac fossa, obturator foramen, sacro-sciatic notch, and sciatic tubers; the presence or absence of a preauricular fossa, which most females possess; and the shape and size of the ring formed by the sacrum and the ossa coxæ. In correctly mounted male and female skeletons note the difference in the inclination of the pelvis as a whole to the axis of the trunk. (Cf. the pelvic girdle with that of quadrupedal forms in this particular and in general proportions.)

# (b) The Free Limb.

Femur (the longest bone of the body, the entire height = 3.7 × length of femur).—Note head with its surface for articulation with the acetabulum; pit (fovea) in the middle of the head for the ligamentum teres which holds the femur in the acetabulum; anatomical neck (coincides with the surgical neck); note variation of the angle between the neck and the shaft at different ages, with nearer approach to a right angle and consequent liability to fracture in old age; greater and lesser trochanters connected by intertrochanteric lines; trochanteric fossa; linea aspera; popliteal

plane; lateral and medial condyles, with articular surfaces for the tibia; lateral and medial epicondyles; intercondyloid fossa; articular surface for the patella.

Tibia.—Note lateral and medial condyles with superior articular surfaces for the condyles of the femur; dorsal and ventral intercondyloid fossæ; intercondyloid eminence; articular surface for proximal end of the fibula; tuberosity; anterior crest; intercosseous crest; medial malleolus; inferior and malleolar articular surfaces for the talus; fibular notch for the distal articulation of the fibula.

**Fibula.**—Note head; apex of head; interosseous crest; lateral malleolus; proximal and distal articular surfaces for the tibia, articular surface of the malleolus.

Patella (a sesamoid bone).—Note apex, base, and articular surface for femur.

Tarsus.—Proximal row, consisting of calcaneus and talus; the navicular bone, interposed between the talus and the distal row; the distal row, consisting of the first (medial), second, and third cuneiform bones, and the cuboid bone. Note that the surfaces for articulation with the tibia and fibula are located upon the talus, which also articulates with the calcaneus, the shelf-like process of which (sustentaculum tali) supports the talus from below. Work out with care the other intertarsal articulations, and the relation of the bones of the distal row to the separate metatarsals (cf. corresponding bones of hand).

Foot.—Note the five digits with ranks of bones as in the case of the hand, metatarsals forming the first rank and phalanges the remaining ones. Note prominent process on the fifth metatarsal. Look for indications, in the forms of the articular surfaces, of less movability of the great toe than of the thumb. Note frequency of fusion of middle and distal phalanges of the little toe, a general human tendency found as often in barefooted races as in those wearing shoes, and hence not due, as often asserted, to the latter condition.

## D. SUGGESTED SUPPLEMENTARY EXERCISES.

1. Identification and classification of the articulations, or arthroses, immovable (synarthroses), slightly movable (amphi-

arthroses), and freely movable (diarthroses), with the various forms of each, particularly the latter group. (Cf. demonstration preparations of articulations, showing arrangement of ligaments.) Record the study of arthroses by appropriate labeling of the drawings already made.

2. Practice in identifying fragments of human bones which bear distinctive features, also whole bones and fragments of bones of other mammals, in which the features are for the most part sufficiently like those of the human bones to be recognizable.

### VI. HISTOLOGY OF MUSCLE TISSUE

#### A. SMOOTH OR UNSTRIATED INVOLUNTARY MUSCLE.

- 1. Tease on a slide a few shreds from a portion of the muscular coat of the intestine of a cat or other mammal (fresh material), which has macerated for 48 hours in 20% nitric acid. Add glycerine and cover. Study the preparation with the compound microscope, and note that each muscle fiber consists of a single elongated, spindle-shaped cell with one granular, elongated nucleus, and with numerous fibrils extending lengthwise through the cytoplasm. Draw a few well-selected examples.
- 2. Study transverse sections (Lab. Sl. Coll.) through the small intestine of Necturus or other amphibian. Note that the muscular coats, both circular and longitudinal, are composed of smooth muscle cells closely packed together with their long axes parallel to each other. Draw a detail showing a longitudinal section of a few adjacent cells (from the circular coat), and one showing a few cells in cross section (from the longitudinal coat).

#### B. STRIATED VOLUNTARY MUSCLE.

From your study of all the following preparations note that a striated or voluntary muscle fiber consists of a long, cylindrical, multinucleate structure, or syncytium, enclosed in a delicate membrane, the sarcolemma, and ending at each end in the case of vertebrates in a conical attachment to a bundle of tendon fibers. Within the sarcolemma and visible only with high power, are innumerable minute fibrils, extending throughout the entire length of the fiber and exhibiting minute cross striations. These are the contractile elements of the fiber, and their cross striations, visible *en masse* even under low power, give the fiber as a whole its cross-striated appearance. Note whether nuclei are scattered throughout the thickness of each fiber or lie only immediately beneath the sarcolemma (cf. rabbit and Necturus in this regard).

1. Tease in a drop of normal salt solution on a slide, a few shreds of voluntary muscle from a recently killed animal, for

example, Necturus, frog, or rat. Cover and examine. Stain under the cover-slip with methylene blue.

- 2. Mount in a drop of glycerine a few teased fibers of muscle, either amphibian or mammalian, which has been preserved in 70% alcohol or 10% formalin. Cover and examine.
- 3. Study transverse and longitudinal sections of rabbit and of Necturus muscle (Lab. Sl. Coll.).

Draw details to show your conception of the structure of a striated muscle fiber, including cross sections of fibers.

## C. STRIATED INVOLUNTARY, OR HEART MUSCLE.

Examine sections of heart muscle and note that although its fibrils show cross striations very similar to those of voluntary muscle, the syncytium is in the form of a reticulum instead of separate fibers, and that the nuclei are relatively large and few. *Draw*.

#### VII. DETAILED ANATOMY OF LIMB MUSCLES

Material.—Full-grown rabbit (or cat). This should be prepared several days, at least, before it is to be used, by the following method: Remove the skin from the entire animal except the feet, and the region immediately surrounding the orifices. Eviscerate the abdomen through a transverse incision. (For convenience in hardening, this transverse incision may be extended to divide the whole body into anterior and posterior halves.)

Harden in a considerable quantity of saturate solution of corrosive sublimate (bichloride of mercury) for 3 days. Wash thoroughly with water. Preserve for subsequent work in 60% alcohol.

Directions for Dissection.—In dissecting muscles, always expose each muscle by removing loose connective tissue coverings and attachments to other muscles, but leave intact as long as possible the muscle itself and its tendinous attachments. Study each muscle in its relationship to others and to the bony skeleton, using for reference, rough skeletal preparations1 of the part studied. Note its form, the direction of its fibers, its origin and insertion, and reason out from this what its work must be. Identify each muscle by its origin and insertion using the tabulated list<sup>2</sup> as a means for naming the muscles of a region after these have been dissected and studied, since often the complete identification must be postponed until other muscles have been worked out. When it is necessary to cut a muscle, make a clean cut transversely through the belly and reflect each end in order to follow out the muscle completely and to expose underlying parts. Never remove a muscle until it has been fully studied and drawn, and then only when necessary to expose deeper parts.

Make a series of carefully labeled drawings to show all the facts determined.

Finally skeletonize the appendage and girdle which you have dissected, by cutting off each muscle from its two attachments

<sup>&</sup>lt;sup>1</sup> The supply of these skeletal preparations is provided for and kept up from year to year by means of those which each class makes upon the completion of this piece of dissection.

<sup>&</sup>lt;sup>2</sup> In case the cat is used instead of the rabbit, Davidson's Mammalian Anatomy with Especial Reference to the Cat will be found helpful.

to the bones, leaving the bones themselves held together by ligaments.

Before discarding the material remove the kidneys and preserve them in 70% alcohol for later study, p. 132.

A. THE ANTERIOR LIMB MUSCLES.

Superficial muscles of the shoulder and chest region

(Identify the dorsolumbar fascia which covers all but the most superficial muscles of the back, and is continuous anteriorly with the cervical fascia.)

Name of muscle	Origin	Insertion
I. Trapezius	From the dorsolumbar fas- cia, and along the mid- dorsal line over the spinous processes of the thoracic and cervical vertebre, and from the occipital protuber- ance of the skull.	Metacromion process, supra- scapular fascia, and dorsal half of spine of scapula.
2. Latissimus dorsi	Partly from the dorsolumbar fascia, partly from the three posterior ribs.	Medial surface of shaft of humerus.
3. Levator scapulæ	Base of the skull.	Metacromion process.
4. Sternomastoideus	Anterior end of sternum.	Mastoid process of skull.
5. Cleidomastoideus	Clavicle.	Mastoid process of skull.
6. Occipitohumeralis	Base of the skull.	Lateral third of clavicle.
7. Cleidodeltoideus	Lateral half of clavicle.	Deltoid tuberosity of humerus.
8: Acromiodeltoideus	Acromion process of scapula.	Deltoid tuberosity of humerus.
9. Spinodeltoideus. (More conveniently studied in connection with the muscles of the scapula.)	Infraspinous fascia, the spine of the scapula, acromion and metacromion.	Lateral surface of humerus distal to its head.
10. Pectoralis major, consisting of many divisions.	Sternum.	Deltoid tuberosity (ridge) of humerus.
Deeper mus	cles of the shoulder and chest	region
II. Pectoralis minor	Sternum.	Clavicle and supraspinous fascia.
12. Rhomboideus major	Spines of anterior thoracic vertebræ.	Vertebral border of scapula
13. Rhomboideus minor	Spines of cervical vertebræ.	Vertebral border of scapula.
14. Rhomboideus capitis	Lateral surface of the skull posterior to the ear.	Vertebral border of scapula
15. Serratus anterior	By digitations from the lateral region of the third to ninth ribs.	Vertebral border of scapula
16. Levator anguli scapulæ	Transverse processes of posterior five cervical vertebræ.	Costal surface of scapula close to its vertebral border
Muscles	on the costal surface of the sc	apula
17. Teres major	Axillary border of scapula	Shaft of humerus near insertion of the latissimus dorsi.

Name of muscle	Origin	Insertion
18. Subscapularis	Whole surface of the sub- scapular fossa.	Smaller tubercle of humerus.
19. Coracobrachialis	Coracoid process of scapula.	Proximal end of shaft of humerus.
Muscles	of the outer surface of the sc	apula
20. Supraspinatus, covered by the pectoralis minor (11).	Whole surface of supraspinous fossa.	Proximal margin of larger tubercle, of humerus.
21. Infraspinatus, covered by the spinodeltoideus (9).	Whole surface of infraspin- ous fossa.	Larger tubercle of humerus.
22. Teres minor	Ventral third of axillary border of scapula.	Larger tubercle of humerus.
	Muscles of the upper arm	
23. Extensor parvus antibrachii	Fascia of the upper arm.	Olecranon.
24. Triceps brachii, of four parts of heads:  (a) Long head	Ventral third of axillary border of the scapula.	Olecranon.
(b) Lateral head	Lateral surface of the shaft of the humerus and lateral epicondyle.	Olecranon.
(c) Medial head	Medial surface of the shaft of the humerus.	Olecranon.
(d) Accessory head	Medial epicondyle of humerus.	Olecranon.
25. Biceps brachii	Anterior edge of glenoid cavity (tendon of origin runs through the inter- tubercular sulcus).	Tuberosity of radius.
26. Brachialis	Lateral surface of proximal portion of humerus.	Radius proximal to insertion of biceps.
Muscles of	the forearm and hand, extense	or group
27. Extensor carpi radialis	Lateral epicondyle of humerus.	Proximal ends of second and third metacarpals.
28. Extensor digitorum communis.	Lateral epicondyle of humerus.	Tendons pass under the annular ligament and are inserted into the middle and distal phalanges of the second to the fifth digits.
29. Extensor pollicis et indicis	Proximal region of shafts of ulna and radius.	Tendon enters the hand with that of extensor digitorum communis (28), divides into two, one of which is inserted into distal phalanx of pol- lex, the other into distal end of metacarpal of index.
30. Abductor pollicis	Lateral surface of shaft of radius.	Metacarpal of pollex.
31. Extensor quarti digiti, extensor quinti digiti, extensor carpi ulnaris.	Three slender muscles arising close together from lateral epicondyle of humerus (the extensor quinti digiti also in part from the shaft of the ulna).	Distal phalanx of fourth digit, fifth metacarpal and proximal phalanx of the fifth digit, and proximal end of fifth metacarpal, respec- tively.

#### Muscles of the forearm and hand, flexor group

Name of muscle	Origin	Insertion
32. Flexor carpi ulnaris	Medial face of olecranon and medial epicondyle of hu- merus.	Pisiform.
33. Pronator teres	Medial epicondyle of humerus.	Middle of medial side of shaft of radius.
34. Flexor carpi radialis	Medial epicondyle of humerus, adjacent to origin of pronator teres (33).	Proximal end of second metacarpal.
35. Palmaris	Medial epicondyle of humerus in association with the superficial head of the flexor digitorum profundus (37).	Lost in palmar fascia, or sheet of connective tissue covering ventral surface of manus, sending off a small slip which is inserted into distal phalanx of pollex.
36. Flexor digitorum sublimis (perforatus).	Medial epicondyle of humerus between the flexor carpi ulnaris and the superficial head of the flexor digitorum profundus (37).	Divides into four tendons which are superficial to the tendon of flexor digitorum profundus, and pass to the manus and ventral faces of the second to fifth digits, where each tendon divides at base of proximal phalanx into two slips which pass one on either side of phalanx to be inserted into proximal end of middle phalanx.
37. Flexor digitorum profundus (perforans) made up by union of four heads:  (a) Superficial head	Medial epicondyle of humerus.	Its tendon spreads out into a broad stout sheath lying immediately dorsal to the tendon of the flexor digitorum sublimis (36); from this sheath five tendons are given off, one to each digit, each passing along the ventral face of the digit to be inserted into its distal phalanx; in the second to fifth digits the tendons pass between the two slips into which the corresponding tendon of the flexor digitorum sublimis is divided.
(b) Middle head	Medial epicondyle of humerus.	
(c) Radial head	Proximal part of flexor surface of the radius.	
(d) Ulnar head	Flexor surface of ulna.	

B. THE POSTERIOR LIMB MUSCLES.

Muscles of the lumbar region

(The muscles of this group lie in the dorsal wall of the abdominal cavity and for the most part pass beneath the inguinal ligament to reach their insertion into the appendicular skeleton. This ligament, which stretches from the anterior spine of the crest of the lilum to the tubercle of the pubis, serves not only as the insertion of portions of the abdominal muscles but also as the origin of the sartorius muscle (14), and it is therefore advisable to postpone the dissection and identification of the lumbar group of appendicular muscles until after the muscles of the medial surface of the thigh have been studied.)

Name of muscle	Origin	Insertion
I. Psoas major	Last three ribs, the ventral surface of the bodies of the last three thoracic and all the lumbar vertebræ.	Into the lesser trochanter of the femur.
2. Quadratus lumborum	Two parts separated by transverse processes of lumbar vertebræ; the inner arising from last five thoracic and all the lumbar vertebræ, the outer from last five ribs, and corresponding transverse processes, and from all the lumbar vertebræ.	Partly into the lumbar vertebræ partly into ventral border of os ilium.
3. Psoas minor	Ventral surface of the bodies of the four posterior lumbar vertebræ.	Pubis by a tendon which acquires a connection with inguinal ligament.
4. Iliacus, continuous with the inner part of quadratus lumborum (2).	Ventral faces of last lumbar and first sacral vertebræ, and medial and lateral sur- faces of the os ilium.	Lesser trochanter of the femur.

Superficial muscles of the hip and thigh regions, lateral and flexor surfaces (The fascia lata which covers over the muscles of this region serves as the origin of some of the more superficial portions of these muscles. It should not be removed but may be slit along the lines of the boundaries of the muscles which lie beneath.)

5. Biceps femoris: (a) Anterior head	Sacral and three anterior caudal vertebræ.	Into the lateral border of a strong and extensive fascia over distal end of femurand proximal end of tibia
(b) Posterior head: Main portion Accessory portion	Posterior part of sciatic tuber. Anterior part of sciatic tuber.	
6. Semimembranosus	Sciatic tuber.	By same fascia as gracilis (15 into proximal part of the medial border of tibia from the distal end of its posterior edge a long tendor passes along the medial side of the lower leg and joins the tendon of the triceps suræ (25).
7. Glutæus maximus	Sacrum, dorsal border of the os ilium, and fascia lata; dorsally its origin is covered by the anterior head of the biceps femoris (5a) ventrally it blends with the rectus femoris (21a).	Slightly distal to the greater trochanter of the femur into the third trochanter.
Dee	per muscles of the hip region	
8. Glutæus medius, covered by glutæus maximus (7).	Dorsal border of os ilium and from sacrum.	Greater trochanter of femur.
9. Glutæus minimus, covered by the glutæus medius (8).	Dorsal border and lateral surface of the os ilium, and from the first sacral vertebræ.	Greater trochanter of femur.
o. Piriformis	Second and third sacral vertebræ.	Greater trochanter beneath the insertion of the glutæus medius, and posterior and dorsal to that of the glutæus minimus.

Name of muscle	Origin	Insertion
II. Quadratus femoris	Sciatic tuber.	Extensor side of shaft of femur, slightly distal to the level of the greater tro- chanter.
12. Obturator internus, and the two gemelli.	Medial aspect of membrane which covers the obturator foramen, and from the medial surface of the pubis and ischium.	Trochanteric fossa.
13. Obturator externus	Lateral aspect of the obturator membrane.	Trochanteric fossa.
Muscles	of the medial surface of the t	high
14. Sartorius	Middle of inguinal ligament.	Blends with the anterior edge of the gracilis (15).
15. Gracilis	Whole length of symphysis pubis.	By a broad fascia which inserts into the proxima part of the medial border of the tibia.
16. Adductor magnus	Posterior edge of ischium and sciatic tuber.	Medial edge of distal end of femur and medial condyle of tibia.
17. Semitendinosus, imbedded in the adductor magnus (16).	Sciatic tuber.	By a long tendon which emerges from the adductor magnus (16), near the distal end of its outer sur- face, and is inserted into the medial condyle of the tibia.
18. Adductor longus	Whole length of symphysis pubis.	Middle third of shaft of femur.
19. Adductor brevis	Anterior end of symphysis pubis.	Shaft of femur.
20. Pectineus	Pubic arch.	Shaft of femur.
Muscle	s of the extensor surface of th	igh
21. Quadriceps femoris, consisting of the following parts: (a) Superficial head of rectus femoris.	Ventral border of the os	By a strong thick tendon,
(b) Deep (short head of rectus femoris partly enwrapped by vastus lateralis.	Posterior part of ventral border of os ilium.	the ligamentum patellæ (in which the patella is embedded), into the crest of the tibia.
(c) Vastus medialis	Neck of the femur.	
(d) Principal (superficial head of vastus lateralis	Neck of femur and greater trochanter.	
(e) Accessory (deep) head of vastus lateralis	Femur close to origin of superficial head.	
(f) Vastus intermedius	Shaft of femur.	
Mus	scles of the lower leg and foot	
22. Tibialis anterior	Lateral condyle and anterior crest of the tibia.	Proximal end of second (apparent first) metatarsal.

Name of muscle	Origin	Insertion
Name of muscle	Origin	Insertion
23. Extensor digitorum communis.	Anterior surface of the distal end of the femur, external to the anterior intercondy- loid fossa, the tendon of origin passing through the capsule of the knee joint.	Distally the muscle divides into four tendons which pass under the annular ligament, and are inserted into the middle and distal phalanges of the four digits.
24. Extensor digiti secondi	Medial surface of proximal end of tibia.	Unites with the first tendon of the extensor digitorum communis (23).
25. Triceps suræ, consisting of:  (a) Gastrocnemius, a large two headed muscle forming the larger part of the calf of the leg. 1. Medial head	Medial epicondyle of femur and medial sesamoid of knee joint.	
2. Lateral head	Lateral epicondyle of femur and lateral sesamoid of knee joint.	the tuber of the calcaneum.
(b) Soleus	Head of fibula.	
<ol> <li>Plantaris (=flexor digitorum sublimis of hand) en- wrapped by the gastrocne- mius (25a).</li> </ol>	Posterior surface of the femur proximal to the lateral epicondyle, and from the lateral sesamoid of the knee joint.	Its tendon divides into four slips, each of which divides at the base of the proximal phalanx into two slips, which pass on either side of the phalanx, and are inserted into the proximal end of the middle phalanx.
27. Popliteus	Lateral epicondyle of femur.	Proximal region of posterior surface of the tibia.
28. Flexor digitorum communis (=flexor digitorum pro- fundus of the hand).	Head of fibula and posterior surface of proximal region of both fibula and tibia.	Four tendinous slips, each of which passes between the two slips into which each tendon of the plantaris (26) divides, and is inserted into the terminal phalanx of the digit.
29. Peroneus longus	Lateral condyle of tibia.	Cuboid.
30. Peroneus brevis	Lateral condyle of tibia.	Proximal end of fifth (last) metatarsal.
31. Peroneus quarti digiti	Shaft of fibula.	Distal end of fourth (apparent third) metatarsal.
32. Peroneus quinti digiti	Lateral condyle of tibia and head of fibula.	Distal end of fifth (last) metatarsal.

#### VIII. HISTOLOGY OF NERVE TISSUES

## A. NERVE CELLS (NEURONES).

1. Smear preparations of gray nerve substance from the spina cord. These are made by applying to a clean cover-slip by means of the point of a scalpel, a bit of the gray matter from the freshly exposed ventral region of the spinal cord of a recently killed mammal.<sup>1</sup> Remove the cover-slip with the adhering nerve substance, place this in contact with another cover-slip, press the two tightly together and then separate them by sliding them apart, thereby spreading the nerve substance in a thin film over the surface of each glass. Dry these smears by holding each carefully in a pair of forceps and passing it three or four times, film side up, through an alcohol flame, allowing a few seconds to elapse between each two successive exposures to the flame. When the film is dry, stain it in a dilute aqueous solution of methylene blue by gently floating the cover-slip, film down, upon the surface of the stain, which may be conveniently used in a watch glass. The process of staining should be continued until a deep purple color is imparted to the film. Rinse in water, dry off the excess of water with filter paper, being careful not to touch the film, and lay the preparations aside with the film up until thoroughly dry. Clear by floating each cover-slip, film down, in a watch crystal of xylol. Place a drop of balsam on the middle of a clean slide and after carefully removing the excess of xylol from the film, place the cover-slip, film down, upon the balsam.

Examine under low power, and amid the general debris look for large, branched nerve cells. Move the slide so as to bring one of these into the center of the field and apply the high power. Note the nucleated cell body or perikaryon; the numerous deeply stained tigroid bodies (Nissl corpuscles) and delicate neuro-fibrillæ in the cytoplasm; the paler nucleus, often irregular in outline

<sup>&</sup>lt;sup>1</sup> Material obtained from a market often proves excellent, even after it has been frozen.

surrounded by a clear circumnuclear zone, and containing always a deeply stained nucleolus. Examine the various cytoplasmic processes and note in general the presence of tigroid bodies in these. A single process, the axone or neurite does not possess these and may thus be distinguished from the remaining processes, the dendrites. Neuro-fibrillæ extend throughout all the processes. Draw a good typical cell, showing the above named parts.

2. Golgi preparations of nerve cells in sections of the cerebral and cerebellar cortex (Lab. Sl. Coll.). These preparations, in which the neurones have been rendered black by the action of silver nitrate, are particularly for the purpose of showing the forms of the various cells and the course of their branches. They are thick preparations and do not as a rule permit the use of the high power objective. In some cases the axone may be distinguished from the dendrites by its smoother surface and its right-angled form of branching (cf. pictures of nerve cells in any good text-book of histology). Look particularly in the cerebral cortex for the characteristic pyramidal cells and in the cerebellar cortex for the Purkinje cells with their flask-shaped perikarya and enormous tree-like dendrites. Draw one or more good typical cells, and identify the types drawn by reference to some text-book of histology.

3. Sections through the cerebral and cerebellar cortex stained to show the details of the cells of the different layers in situ (Lab. Sl. Coll.). Study as many such sections as the time and material permit, identifying the layers and their characteristic cells by use of any good reference or text-book of histology. Note particularly the characteristic pyramidal cells of the cerebral cortex, and the Purkinje cells of the cerebellar cortex. Draw as much detail as

you are able to identify and understand.

## B. NERVE FIBERS, THE PROLONGED PROCESSES OF NERVE CELLS.

From the study of the following preparations note that there are two kinds of fibers, in both of which the essential part is the conspicuous, slender, non-nucleated column of protoplasm, known as the axis cylinder, which lies on the middle of the fiber and is in reality the much elongated process of some nerve cell. The conspicuous structures in the preparations are the sheaths of these fibers, in accordance with which the fibers are classified.

Medullated Nerve Fibers.—These are the most abundant, and possess a double sheath, the outer a thin one of connective tissue cells known as the sheath of Schwann or the neurilemma, the inner one a thicker fatty layer (blackened by osmic acid) known as the medullary or myelin sheath, or the white substance of Schwann. The medullary sheath is not continuous, but is interrupted at regular intervals, the places of interruption appearing as constricted regions or nodes, and the length of fiber between two nodes forming an internode. Note that the sheath of Schwann is continuous over the nodes, and that it has one nucleus (which often bulges out conspicuously) for each internode of length. Note the double contour of medullated fibers in cross section, also the very great range of variation in size shown by the cross sectional areas.

Non-medullated Nerve Fibers.—These are fewer in number than the medullated type, and are very inconspicuous. Each has a single sheath, the neurilemma, or sheath of Schwann, with here and there elongated nuclei which, as they bulge out from the side of the fiber, give it a peculiar wavy contour.

Draw a sufficient length of nerve fiber of each kind, to show the above structures. Draw cross sections of medullated nerve fibers, showing the two sheaths and a nucleus of the outer sheath.

- I. Fresh teased preparation of nerve fibers made by teasing upon a dry slide a short length of small nerve taken from a recently killed animal (Necturus, frog, or some small mammal). During the teasing process the preparation should be kept from too rapid drying by breathing gently upon it, and the process of teasing should be continued until the separated fibers have dried down sufficiently to stay in place on the slide. Add a drop of physiological salt solution and cover.
- 2. Teased preparations of nerve fibers previously hardened, and stained in hæmatoxylin (which stains the axis cylinder, the nuclei of the sheath, and the supporting, non-cellular meshwork of the medullary sheath), and mounted in Canada balsam (Lab. Sl. Coll.).

- 3. Teased preparations of nerve fibers which were stained, while fresh, with osmic acid (which blackens the medullary sheath), and subsequently mounted either in glycerine or in Canada balsam (Lab. Sl. Coll.). Note in addition to the above described features, the numerous oblique incisions in the medullary sheath.
- 4. Cross sections of nerve fibers, stained with hæmatoxylin (Lab. Sl. Coll.). In case these sections include the whole nerve, note the connective tissue sheath of the nerve as a whole, the perineurium, and the more delicate sheaths of the component bundles, the endoneurium.
- . 5. Cross sections of nerve fibers which were previously treated, while fresh, with osmic acid (Lab. Sl. Coll.).

## C. NERVE ENDINGS (Lab. Sl. Coll.).

## 1. Motor Nerve Endings.

Motor End Plates in Muscle Fibers.—Note the deeply stained axis cylinders of the bundles of fibers which enter the muscle mass; follow single fibers to their terminations in flat discs (end plates) one of which is applied to each muscle fiber. Note whether a single nerve fiber ever divides and supplies more than one muscle fiber. *Draw*.

Study a single disc with great care under the high power. Note that the axis cylinder of the nerve fiber breaks up into a network or reticulum to form the disc. *Draw details to show these points*.

## 2. Sensory Nerve Endings.

Muscle Spindles, in Muscle Fibers.— Note that each spindle is differentiated from portions of several muscle fibers. Work out with care under the high power, the relation of the nerve fibers to the muscle spindle. Draw a single spindle showing details.

Pacinian Corpuscles, in the Areolar Tissue of Mesentery or of the Pancreas of the Cat.—Note that the end of the nerve fiber is covered by numerous concentric wrappings of connective tissue, which serve as a mechanical means for transmitting stimuli to the nerve ending. Select slides in which the Pacinian corpuscle may be seen cut transversely and study the arrangement of these parts. Select also a good longitudinal section through the middle of a corpuscle. Draw each view.

Taste Buds, in the Epithelium of the Tongue (sections through the foliate papillæ of the rabbit are recommended).—Note that the surface of the tongue is covered by a thick epithelium consisting of many layers of cells closely packed together. Note the difference in shape of the cells of the superficial and deeper layers. the epithelium which covers certain conspicuous folded regions (foliate papillæ) identify the taste buds, each of which is a cluster of much elongated cells with their long axes at right angles to the surface of the fold. These differentiated epithelial cells are of two sorts, gustatory or taste cells and supporting cells. The gustatory cells, within the taste bud, are surrounded by the end arborizations of nerve fibers, which are rendered visible only by special methods of staining. Other branches of nerve fibers end among the cells between the adjoining taste buds. At its outer end each gustatory cell possesses a cilium which projects slightly from the surface through the opening of the organ, known as a taste pore. Draw a single good section through a taste bud, showing the relation of the cells to this pore.

Olfactory Epithelium (teased or dissociated preparations are recommended; also sections of olfactory epithelium of some amphibian, stained with iron hæmatoxylin or other stain especially adapted to bring out nerve cells and fibers).—The olfactory organ is peculiar in that there are present among certain tall columnar epithelial cells, other tall cells which are nervous in nature. Each of these has a short process (dendrite) which extends to the free surface, and another much elongated process (neurite) which is an olfactory nerve fiber and extends to the olfactory lobe of the brain. In the preparations supplied distinguish the two sorts of cells and draw a few of each, either in place or dissociated.

The Retina<sup>1</sup> (slides showing vertical sections through the retina, e.g., of the rabbit and the frog).—Under the low power note that the staining methods used have disclosed the fact that the retina consists of many layers of structures of different thickness in different regions. Examine a carefully selected region under high

<sup>&</sup>lt;sup>1</sup> This study of the retina may conveniently be postponed until after the study of the eyeball (p. 88).

power. Beginning with the most external layer, the layers of the retina are named and constituted as follows:

- i. The pigment layer (outside of this, the chorioid coat, also pigmented, and the sclerotic, are present in some of the preparations).
- ii. The layer of rods and cones, their apices directed toward the pigment layer.
- iii. The external limiting membrane, a delicate line, scarcely visible, and consisting mainly of the branches of the neuroglia, *i.e.*, supporting cells.
- iv. The outer nuclear layer, a conspicuous layer exhibiting many deeply stained nuclei which are, for the most part, those of the nerve cells of which the rods and cones are the highly specialized processes. Among these, and indistinguishable in these preparations from them, are nuclei of neuroglia cells.
- v. The outer molecular layer, finely granular in appearance, because it consists of the cut ends of the delicate branches of the cells of the nuclear layers between which it lies.
- vi. The inner nuclear layer, consisting of bipolar nerve cells, the deeply stained nuclei of which give the name to the layer.
- vii. The inner molecular layer, with a structure similar to that of the outer molecular layer.
- viii. The ganglion cell layer, consisting of ganglion cells the rather large nuclei of which indicate the extent of the layer.
  - ix. The nerve fiber layer, consisting of the fibers of the optic nerve, which are for the most part neurites of the cells of the ganglion cell layer. These fibers converge from various parts of the retina toward the point of exit of the optic nerve on its way to the brain (i.e., the blind spot).

Compare these slides with the pictures in the various textbooks of histology, and with the laboratory charts. Draw a diagram from the slide showing the location of the various layers and any details which you can see clearly.

The Spiral Organ of Corti<sup>1</sup> (slides showing sections through the cochlea of the ear of the pig are recommended).—Locate in the section a transverse section through a turn of the cochlea. Study this under low power and note the rounded form of the bony tube; the triangular form of the section through the ductus cochlearis, in which the spiral organ of Corti lies supported on the lamina basilaris (basilar layer) which stretches between the bony shelf (the lamina spiralis ossea) and the opposite wall of the tube; the scala vestibuli, separated from the ductus cochlearis by a delicate membrane usually torn in the sections (the membrana vestibularis Reissneri); the scala tympani, separated from the ductus cochlearis by the lamina basilaris; the membrana tectoria, supported from the lamina spiralis ossea and overhanging the spiral organ; the spiral ganglion of the cochlea, lying in the lamina spiralis and sending nerve bundles beneath the lamina spiralis to reach the spiral organ of Corti.

Under the high power study the details of the spiral organ of Corti. Note within this, the rounded triangular section of a cavity which accompanies the spiral organ through the windings of the cochlea, and is known as the tunnel. The base of this rests on the lamina basilaris, and the two sides are formed by the rods of Corti. The larger portion of the spiral organ of Corti lies on the outer side of the tunnel and consists of numerous tall epithelial cells specialized from the general epithelial lining of the ductus cochlearis, and supporting other more highly specialized cells, known as the outer hair cells from the fact that they bear on their exposed surface a group of short hair-like processes. On the inner side of the tunnel is a single row of inner hair cells, similarly supported by adjoining epithelial cells. These hair cells are the structures which are stimulated by the sound vibrations and transmit the stimuli to the nerve endings (visible only with certain methods of staining) of the auditory nerve fibers. Show by diagram made from your study of the cross section the general features of the cochlea and of the spiral organ of Corti.

<sup>&</sup>lt;sup>1</sup> This study of the Organ of Corti may conveniently be postponed until after the dissection of the ear (pp. 92-94).

# IX. STUDY OF THE SPINAL CORD AND PERIPHERAL NERVOUS SYSTEM

#### A. GROSS ANATOMY.

Study demonstration dissections of cat or other small mammal 1 showing the whole spinal cord and spinal nerve roots (1) in situ, and (2) removed from the neural canal. Examine, also, short lengths of the spinal cord of some larger mammal (calf, lamb, or pig), with the dura mater removed in order to show better the external surface of the cord and the relation of the nerve roots to it. From these various preparations note the relation of the dura mater, arachnoid, and pia mater to the cord and to the nerve roots; the general form of the cord with its cervical and lumbar enlargements and its gradually tapering posterior end forming the filum terminale which, together with the more posterior pairs of spinal nerves, forms the tail-like structure known as the cauda equina; the ventral median fissure, and the dorsal median sulcus; the less sharply marked dorsal and ventral lateral sulci, the lines along which the dorsal and ventral nerve roots. respectively, leave the cord; the segmental arrangement of nerve roots corresponding to the intervertebral spaces, the more anterior roots passing out almost at right angles, while the more posterior ones have a very oblique posterior course; the presence of a ganglion on the dorsal (sensory) root; the point of union of the two roots. Draw.

From more detailed dissections of a limited region, study the dorsal and ventral rami of each nerve, the former distributed mainly to the skin and skeletal muscles, the latter to the deeper

¹ New born or very young kittens furnish excellent material for these preparations. They should be skinned while fresh, and the roof of the cranial cavity removed. The abdomen also should be laid open by a longitudinal slit. They should then be hardened for several weeks in 5% formalin made up with 30% alcohol (see p. 75) instead of water. When thoroughly hardened they may be washed out for a day or two in water and then dissected. They may be kept from year to year in 70% alcohol, or in 5% formalin.

muscles and viscera; and the **communicating ramus** between each spinal nerve and the corresponding ganglion of the sympathetic trunk. *Draw*.

Examine other demonstration preparations made by dissecting from the ventral side. In making these it is necessary to remove much of the ventral musculature, and to lay open the thoracic, abdominal and pelvic cavities and remove most of the contained viscera, as well as the dorsal portion of the parietal serous membranes. The following are the more important structures to be seen:

The course of the vagus nerve (and its accompanying sympathetic trunk), from its ganglion, which is located immediately posterior to the jugular foramen, through the whole length of the neck and the thoracic cavity, where it gives off branches to heart and lungs, and then continues through the diaphragm to the surface of the stomach where it enters into the formation of a plexus.

The phrenic nerve which arises from a plexus formed by the ventral rami of the cervical nerves, and proceeds through the thoracic cavity passing over the surface of the heart to innervate the diaphragm.

The brachial plexus formed from the ventral rami of the last three cervical and the first thoracic nerves, and located in the region between the anterior portion of the thoracic wall and the axilla. Follow ulnar, median, and radial nerves into the forearm and hand, noting the presence of both cutaneous and muscular branches.

The lumbosacral plexus formed from the ventral rami of the posterior lumbar and anterior sacral nerves, and located in the dorsal wall of the pelvic cavity. Among its many branches may be distinguished the femoral branch which passes under the inguinal ligament to the medial side of the thigh, and the very large sciatic branch which bends about the greater sciatic notch and passes to the back of the leg dividing in the lower leg into medial and lateral popliteal branches. Note the presence of both muscular and cutaneous branches.

The delicate sympathetic trunk located upon each side of the mid-line back of the serous membrane in the dorsal wall of the

whole cœlomic cavity. Each trunk consists of segmentally arranged chain ganglia joined together serially by slender connectives. Note that the sympathetic trunks extend through the neck region accompanying the vagus nerves, but that there are only three pairs of cervical ganglia. The most anterior of these lies close beside the ganglion of the vagus, and the middle one is located in close proximity to the very large stellate ganglion, the most posterior of the series, immediately anterior to the first rib. Each stellate ganglion gives off a branch to the vagus of that side and another branch which joins one from the vagus to be distributed to the plexus of the heart.

Note that the lower thoracic ganglia give off nerves, among them the greater and lesser splanchnic nerves which connect with the cœliac ganglion posterior to the stomach.

Draw such details of the preparation as you are able to see clearly and to understand.

#### B. STRUCTURE OF THE SPINAL CORD.

r. Macroscopic Study.—With a wet section knife make a clean, thin transverse section of a piece of well hardened spinal cord (10% formalin followed by 70% alcohol has been found to give good results). Place one or more of these in water in a clear glass watch crystal and examine it over a black background with a dissecting microscope, for the general topography of the cord. For comparison examine macroscopically over a white background, stained transverse sections (Lab. Sl. Coll.) through various regions of the cord, some of which show sections also of the spinal ganglia. In each of these preparations note whether or not the dura mater is present and its relation to the nerve roots and ganglia, when these also are present in the section. Make out the following points in the general topography thus studied:

The median ventral fissure and the less conspicuous median dorsal sulcus, both reaching nearly to the center of the section; the less sharply marked dorsal lateral and ventral lateral sulci, and the relation of these to the nerve roots.

The section through the gray nerve substance which presents an H shaped area with the lumen of the canalis centralis lying

in the cross bar of the H. The dorsal column of gray substance forms the dorsal region of the upright stroke of the H, the region opposite the cross bar is the lateral column, and the rounded region ventral to the cross bar is the ventral column. The latter, unlike the more pointed dorsal column, fails to reach the surface of the cord.

. The three funiculi of white substance upon each side, the dorsal one located between the median dorsal sulcus and the dorsal column of gray substance, the lateral one located between the lateral surface of the cord and the lateral boundary of the gray area, and the ventral one located between the ventral column of gray substance and the median ventral fissure.

Compare sections through as many levels of the cord as are afforded by the material, and note the varying shapes, and the different proportionate amounts of the gray and white substance at different levels. Draw a section through two or more of the levels thus studied and show the general topography in each, noting in all cases the indefiniteness of the line separating the gray from the white substance.

2. Microscopic Study.—Examine, mainly under low power, several sections of the spinal cord (Lab. Sl. Coll.), and having established in each case the direction of the section with relation to the long axis of the cord, identify the following structures:

The Meninges (dura mater, arachnoid, and pia mater).— Determine how many of these are present in each section, and the relation of each to the surface of the cord, the small root bundles (fila radicularia), and the ganglia, if these are present.

The Epithelial Layer (ependyma) lining the canalis centralis, and any structures such as Reissner's fiber, lying in the lumen.

The Perikarya of Neurons in the Gray Nerve Substance.—In which column are these especially large and conspicuous? Where are they small and few? Are they at all grouped and where are such groups found? Look for sections which show the nucleus dorsalis (Clark's column) in the dorso-medial region adjacent to the cross bar of the H.

Fibers Extending from the Gray to the White Substance or vice versa.—Look especially for those which go to make up the

fila radicularia of the nerves, and determine their relation to the gray nerve substance.

Commissural Fibers.—Note that these lie either within the gray substance of the cross bar of the H (gray commissure), or ventral to the gray substance (white commissure). Determine so far as possible the connections which these make.

The Longitudinal Bundles of Fibers (Fasciculi).—Note that these make up the bulk of the white funiculi, and are here cut transversely. Note, under high power, that these fibers are medullated, while the neurilemma is absent and a sheath of areolar tissue takes its place. Compare with fibers seen in sections of nerve roots outside of the cord.

Nerve Roots and Ganglion.—The general course of the dorsal root bundles of medullated fibers into and through the ganglion, between the groups of perikarya of sensory neurons. Why do the sections of the latter have rounded outlines? Are any of these cut through a process? Note the sheath of supporting cells around each perikaryon. Note the relation of the ventral root bundles to the ganglion.

Select one good characteristic section, passing through a ganglion if possible, make of it as large an outline drawing as your page allows, to show the general topography as seen under the low power, and fill in in their proper regions the details of perikarya and fibers as studied under the high power.

#### X. THE PRIMITIVE VERTEBRATE BRAIN

The dogfish, a small species of shark, represents one of the most primitive groups of fishes, and its brain shows the simple form and arrangement of parts found in early embryos of higher vertebrates. It thus furnishes the key to the interpretation of brain structure in higher forms such as the mammals.

#### A. DORSAL ASPECT OF THE DOGFISH BRAIN IN SITU.

First, remove the skin, then carefully slice away the cartilage which forms the brain case (the animal has no bones) until the entire brain is exposed. The cartilage is translucent, and, if the dissection is done with care, before the nerves are reached they may be seen passing through the foramina and they may thus be preserved in connection with the brain.

Study and draw the brain from the dorsal side, in situ, identifying the following brain vesicles and their parts:

- I. Telencephalon.—Olfactory lobes, cerebral "hemispheres."
- 2. Diencephalon.—Optic thalami, epiphysis (pineal body).
- 3. Mesencephalon.—Optic lobes.
- 4. Metencephalon.—Cerebellum, with a median and two lateral lobes (flocculi).
  - 5. Myelencephalon.—Medulla oblongata.

# B. MEDIAN SAGITTAL SECTION OF THE DOGFISH BRAIN IN SITU.

This section may be made by a single stroke by means of the wet blade of a sharp thin knife or a section cutter through the median plane of the entire head. Study the brain as it appears in median sagittal section, identifying the following features, and recording the facts learned by means of a suitable drawing.

**1. Telencephalon.**—Note the thickening, the corpus striatum, in the floor on each side, and the thinness of cerebral roof, or pallium, which forms the cerebral hemispheres. Each cerebral hemisphere contains a ventricle, the first and second, and these

communicate in the midline through the foramen interventriculare with the third ventricle.

- **2. Diencephalon.**—Note the epiphysis (or pineal body) above, the hypophysis (or infundibulum) below, and the lateral thickenings, the optic thalami. The cavity is the third ventricle.
- 3. Mesencephalon.—Trace the cavity, the aqueduct of the cerebrum, through this, and note that the optic lobes are dorso-lateral thickenings of the walls.
- 4. Metencephalon.—Note its dorsal outpushing, the cerebellum.
- 5. Myelencephalon.—Note the wide fourth ventricle, and its thin roof.

## C. COMPARATIVE STUDY OF BRAINS OF OTHER VERTEBRATES.

Examine other available demonstration dissections, such as the brain of some amphibian (Necturus or frog), and the brain of a reptile (turtle), a bird, and a primitive mammal (rodent), and identify the various brain vesicles and their more important features.

Draw as many as time permits.

#### XI. THE MAMMALIAN BRAIN

Material.—Each student should be provided with the head of a calf or sheep, obtained in as fresh a condition as possible from the abattoir. The head should be cut off well into the neck region, and the tongue left in place. The material is less expensive and more easily handled if the head is skinned, but at least one head should be sent with the skin on for the demonstration of external features and landmarks. The implements needed for the work are in addition to the usual dissecting instruments, a small saw, a ¾ inch carpenter's chisel, a wooden mallet, and bone forceps.

Preparation of Material.—The specimen should be thoroughly washed under running cold water, and if it has been frozen it should be thawed before beginning the dissection. Make a rapid examination of the head externally, noting the relative position of various features, and identifying skeletal landmarks. Identify the structures shown in the cross section of the neck, and draw a diagram recording these.

In case the specimen is not already skinned, remove the skin except around the mouth, the distal region of the nose, and the eyes, using for the purpose very sharp scalpels. The external ear may be removed with the skin. Note the adhesion of the skin to the frontal bone in the region medial to the superciliary arches where in the male the horns may be seen in various stages of growth. Remove the muscles from the lateral and posterior surfaces of the cranium, thereby exposing the temporal ridges and the occipital protuberance. Mark out with the scalpel lines along which to saw to remove the roof of the cranial cavity as follows: (1) an oblique line upon each side medial to and parallel with the superciliary arches, meeting each other in the midline anteriorly, and ending posteriorly just lateral to the temporal ridge; (2) an oblique line upon each side from the posterior end of the first line to the occipital foramen just medial to the occipital condyle. Use a small saw and take care to hold it at right angles to the surface of the bone. Take care not to saw too deeply and thus injure the surface of the brain which lies just beneath the bone. After sawing along the lines marked out, gently pry at various points with a chisel to determine if there are any regions which the saw has not completely separated, and, if such regions are found, cautiously chisel through them by light taps of the wooden mallet. Finally remove the bony roof by prying it up and detaching the adherent regions of the dura mater from its inner surface. This will expose the brain in situ covered by the dura mater. If there are portions of the roof which overhang the brain laterally, carefully chip these away with the bone forceps.

From the exposed surface of the brain remove the dura mater including the falx cerebri, that thickening of the dura which dips into the longitudinal fissure between the two cerebral hemispheres, and the tentorium which lies in the transverse fissure between the cerebrum and the cerebellum. Beneath the dura mater may be seen the pia mater with its plexus of blood vessels. The arachnoid layer which lies between is too poorly defined to appear as a distinct membrane.

To remove the brain from the cranial cavity, gently loosen it from the dura mater on all sides by means of the wet flat handle of a scalpel. Make sure that the tentorium is wholly removed. Lift the anterior end of the brain with care and, reaching in to the extreme anterior region of the floor of the cranial cavity with the wet scalpel handle, detach the olfactory lobes from the cribriform plate of the ethmoid upon each side. The anterior region of the brain may now be lifted up from the floor of the cavity. Then reach still farther back beneath the brain and, with a very sharp scalpel or with scissors, cut the optic nerves as far as possible from the ventral surface of the brain. If the head is now held in a vertical position with the nose pointed upward, gravitation will cause the brain to drop backward sufficiently to enable one to look in at the anterior end between the ventral surface of the brain and the floor of the cavity and see the stalk of the hypophysis, the carotid arteries, and the various pairs of cranial nerves which must be severed successively. As the process continues, the brain will fall farther and farther back and its weight should be supported

by the hand. When it is finally free, place it in a deep dish of water, and examine it in its fresh condition.

Finally harden it, for at least a week, in a mixture of alcohol and formalin (5% formalin in 30% alcohol), taking care to rest it upon cotton and not allow it to come in contact with the walls of the container. It is advisable that only one or two specimens should be hardened in a single container, and there should be at least a liter of the fluid for each specimen (i.e., 300 c.c. of 95% alcohol, 650 c.c. of water, and 50 c.c. of formaldehyde). Some of the hardening fluid should be gently forced by means of a syringe into the cavities of the brain through the opening in the stalk of the hypophysis upon the ventral surface.

For the subsequent study of the brain it should be examined and dissected under water, and, after it is thoroughly hardened, it may be kept in water for several days without deleterious results.

Preserve the head from which the brain was removed in 5% formalin.

## A. EXTERNAL FEATURES.

Block out a general outline of the whole brain  $(x_2)$  in very light lines from dorsal, lateral, and ventral aspects, and add to each drawing the various features as they are identified.

1. Cerebrum (Telencephalon, Diencephalon, and Mesencephalon)

Dorsal Aspect.—Note that the roof of the telencephalon has become greatly thickened to form the two largest and most conspicuous portions of the brain, the cerebral hemispheres, while the real anterior ends of the telencephalon, the slender olfactory lobes, are practically concealed from view. Note the deep median longitudinal fissure, separating the two hemispheres from each other; the frontal and occipital poles of the cerebral hemispheres, and the general division of each hemisphere into regions corresponding to the bones which form the cranial walls, and hence known respectively as the frontal, parietal, occipital, and temporal lobes. Note further that the convoluted appearance of the surface of the cerebral hemispheres is due to depressions, sulci, of varying depth, bounded by elevations or gyri. Note to what extent these are bilaterally symmetrical in arrangement. The sulci, the deeper of which are called fissures, may be followed

out by carefully removing the arachnoid, pia mater, and blood vessels which fill them. The most conspicuous of these upon the dorsal surface is the central sulcus of Rolando, which is at right angles to the longitudinal fissure and partly separates frontal from parietal lobes; the upper end of the lateral cerebral fissure (fissure of Sylvius), mainly latero-ventral in location, which partly separates frontal from temporal lobes may also be seen. Note the general direction and arrangement of gyri and sulci in each area thus mapped out, and determine to what extent this corresponds to the arrangement in the human brain (cf. reference books).

By gently removing the arachnoid from the longitudinal fissure, and pressing the cerebral hemispheres apart, the white surface of the corpus callosum may be seen extending across the midline between the two hemispheres. Posterior to this is located the small median outpushing of the roof of the diencephalon, the pineal body (epiphysis), the disclosure of which is made possible by the removal of the arachnoid from the transverse fissure. This allows the cerebellum to be bent gently backward thus making it possible to see also the four rounded eminences, or corpora quadrigemina, which form the dorsal portion of the mesencephalon. The pineal body lies in the depression between these and the cerebral hemispheres.

Lateral Aspect.—Note the deep lateral cerebral fissure which crosses the lateral surface of the hemisphere vertically, thus forming the boundary between the frontal and the temporal lobes, while the supramarginal and angular gyri of the inferior parietal lobe arch about its upper end. By gently pressing forward the anterior wall of the lateral fissure, which is known as the operculum, it is possible to disclose at the bottom of the fissure a deep region of the convoluted surface of the cerebrum known as the insula, which will, however, be more plainly seen in the subsequent dissection. At its lower end the lateral fissure meets at right angles the rhinal fissure, which extends longitudinally the whole length of the cerebral hemisphere and may be better studied from the ventral aspect.

Ventral Aspect.—Note the conspicuous rhinal fissure which crosses at right angles the ventral end of the lateral fissure, and

continues posteriorly across the temporal lobe, forming the lateral boundary of the hippocampal gyrus; the olfactory lobes, and the olfactory tracts, which may be seen as whitish streaks diverging as they run posteriorly from the olfactory lobes. In the **diencephalon** note the optic chiasma in which the optic nerves come together and cross before continuing as the optic tracts which disappear from view laterally beneath the overhanging edges of the temporal lobes of the hemispheres; immediately posterior to the chiasma, the cut surface of the hollow stalk of the hypophysis; more posteriorly, a rounded elevation, the mammillary body.

In the **mesencephalon** two large bundles of fibers, the peduncles of the cerebrum, may be seen to diverge from each other and disappear under the temporal lobes of the hemispheres.

## 2. Rhombencephalon (metencephalon and myelencephalon).

Dorsal Aspect.—Note the thickened roof of the metencephalon, the cerebellum, consisting of a median lobe, or vermis, and two lateral lobes, all with the surface much convoluted. Extending posteriorly from beneath the cerebellum is the myelencephalon, or the medulla oblongata, the thin vascular roof of which (the tela chorioidea posterior) may be easily removed, exposing the interior of the fourth ventricle of the brain; note that the two narrow fiber tracts, the dorsal funiculi, which run longitudinally on each side of the dorsal median fissure of the posterior part of the medulla, and the two larger, laterally situated tracts, the lateral funiculi, are directly continuous posteriorly with the corresponding funiculi of the spinal cord.

Ventral Aspect.—Note the floccular lobes of the cerebellum upon each side; the transverse band of fibers, the pons (Varolii), which connects the two sides of the cerebellum and thus forms the floor of the metencephalon; posterior to the pons, in the myelencephalon, a trapezoid body may be seen on each side; while on each side of the midline between the trapezoid bodies and extending posteriorly the whole length of the medulla are the two pyramids, continuations of the two ventral funiculi of the spinal cord and separated by the ventral longitudinal fissure; note that in the posterior region, bundles of fibers cross from each pyramid to the other (decussation of the pyramids); posterior to the

trapezoid bodies the pyramids are flanked laterally by the rounded elevations known as the olivary bodies.

Nerve Roots.—(These are variously made up of sensory and motor components, indicated below respectively by the letters S and M. In certain cases, however, in which a nerve is designated as motor, small sensory components may also be present.)

Olfactory (S) from the olfactory lobes. These consist of many small bundles which are always torn from the olfactory lobes in removing the brain.

Optic (S) from the optic chiasma.

Oculomotor (M) appearing from the ventral surface of the cerebral peduncles.

**Trochlear** (M) appearing laterally between the mesencephalon and the metencephalon, but arising from the dorsal surface at the posterior border of the mesencephalon.

Trigeminal (M) appearing from the lateral border of the pons.

**Abducent** (M) appearing from the lateral boundaries of the pyramids slightly posterior to the pons.

Facial (S and M) from the latero-posterior borders of the trapezoid bodies.

Acoustic (S) appearing laterally posterior to the pons.

Glossopharyngeal (S and M) lateral to the olivary bodies.

Vagus (S and M) from the lateral surface of the medulla posterior to the glossopharyngeal.

Accessory (M) from many roots along the lateral surface of the spinal cord and medulla, these roots uniting and extending anteriorly as far as the root of the vagus, which they then accompany out of the cranial cavity.

Hypoglossal (M) from the posterior region of the medulla along the lateral margins of the pyramids.

## B. INTERNAL STRUCTURE.

## 1. Median Sagittal Section.

Place the brain with the ventral surface up upon a layer of cotton on the table, and, using as a guide the midventral fissure,

<sup>1</sup> For convenience of description we may speak of both sorts of nerve roots as coming from the brain but in reality only the motor components actually originate from the brain, while the sensory ones grow into the brain from ganglia or from the sense organs themselves.

make a median sagittal section through the entire brain by a single carefully directed stroke of the wet blade of a long thin sharp knife. Not more than half of the supply of specimens should be used for this purpose, and usually fewer will suffice since two students may conveniently study one good section.

The Cavities of the Brain.—The brain is a hollow organ, the walls of which have undergone repeated folding and thickening, and the cavity of which is continuous with that of the spinal cord, the canalis centralis. The inner surface of the cavity, covered by a smooth epithelium, the ependyma, should be carefully distinguished from the cut surfaces of its walls. The cavities are known as ventricles, four in number and disposed as follows: The first and second (to be demonstrated by later dissection) in the two cerebral hemispheres, i.e., in the telencephalon, the third in the diencephalon, connected with the first and second by a channel known as the foramen interventriculare. The third ventricle is connected with the fourth ventricle, which is located in the rhombencephalon, by the aquæductus cerebri, which passes through the mesencephalon. The first and second ventricles are separated from each other by a thin median partition, the septum pellucidum, triangular in outline, and bounded above by the corpus callosum and below by a curved longitudinal bundle of fibers known as the body of the fornix. The anterior boundary of the third ventricle appears as a thin lamina, the lamina terminalis, extending ventrally from below the anterior end of the fornix to the optic chiasma. The roof of the anterior part of the third ventricle is very thin and is known as the epithelial chorioid lamina, through which the anterior chorioid plexus (blood vessels) pushes its way to reach the inner surface of the third ventricle. The extensive, thin roof to the fourth ventricle is revealed by carefully lifting up from contact with it, the overhanging thickened portion which forms the cerebellum. It consists of the anterior medullary velum, which lies anterior to the junction of the cerebellum with the medulla, and the posterior medullary velum, which lies posterior to the cerebellum. Through the latter velum the posterior chorioid plexus reaches the interior of the fourth ventricle.

Other Important Features Shown in the Sagittal Section.— The exposed wall of the longitudinal fissure formed by the medial surface of the cerebral hemisphere with its various gyri, the most definite and conspicuous of which is the gyrus cinguli which follows the dorsal curve of the corpus callosum; the dorsal and ventral outpushings of the third ventricle, which form the pineal body (epiphysis) and the hypophysis respectively (the latter, however, usually broken off in removing the brain from the cranial cavity); the massa intermedia, a soft mass lying in the middle of the third ventricle and appearing here as a cut area section, circular in outline; the cut surface of the cerebellum, with its tree-like arrangement (arbor vitæ) of white nerve substance (bundles of nerve fibers) surrounded by the gray cortex; the commissures, i.e., bundles of fibers connecting the two halves and hence cut transversely here. These are (a) the corpus callosum, which connects the two cerebral hemispheres, (b) a portion of the fornix just ventral to the posterior end of the corpus callosum, (c) the optic chiasma, (d) the anterior commissure, very small, dorsal to the optic chiasma, and connected with it by the lamina terminalis, (e) the posterior commissure at the dorso-posterior limit of the third ventricle, (f) the pons.

Draw the median sagittal section showing the cavities and other features, and making very clear the distinction between external surfaces, internal surfaces, and cut surfaces.

## 2. Dissection of the Cerebral Hemispheres.

Throughout this dissection draw details to show facts which you learn.

For this, and the subsequent dissections, use preferably a whole brain, supplementing the material as needed by the half brains obtained by making the median section. Orient the dissection by frequent reference to the external features of the brain and to the median sagittal section. Carefully cut away the dorsal surface of the cerebral hemispheres by making thin horizontal slices with a sharp wet razor or scalpel. While making this dissection note the distribution of white and gray matter, and latter forming a thick external layer, the cerebral cortex. Note that there are small bundles of fibers which extend beneath the

cortex from one gyrus to another, while other bundles converge medially to enter into the formation of the corpus callosum, and still others take a more ventral course which will be further demonstrated later. With great care dissect down to the level of the surface of the corpus callosum, changing the direction of the sectioning as is needed to follow the curve of its upper surface and to trace it laterally into each cerebral hemisphere. If sufficient care is used it will be possible to disclose the delicate lateral and medial striæ which extend in an antero-posterior direction along its free surface. By scraping or tearing lightly with the forceps, the transverse direction of the bundles of fibers of which the corpus callosum is composed may be demonstrated.

With great caution continue the horizontal sectioning until by cutting through the lateral regions of the corpus callosum, in each hemisphere, the central part of each lateral ventricle is laid open. Finally cut through and remove the body of the corpus callosum leaving only the anterior bundles of transverse fibers which form its genu, and the posterior bundles which form the splenium, and exposing the cut upper edge of the septum pellucidum, the relation of which as a thin vertical partition between the two lateral ventricles will now be evident. Locate again the external surface of the insula and in the subsequent dissection do not slice away any of its cortex.

Continue the dissection by cutting narrow wedge-shaped portions of the cerebral hemisphere away as need be to remove the roof of the anterior cornu, and of the extensive descending cornu, and thus lay bare the whole floor of the ventricle. Study the whole cavity and its relationships with great care. Note that the central part lies near the midline and was roofed over by the corpus callosum, while the septum pellucidum forms its medial wall, and a rounded eminence, the corpus striatum, lies in its floor and lateral wall. Note that the lateral wall is very thick and that the convoluted cortical surface of the insula forms the external boundary of this lateral wall. The anterior cornu of the cavity curves around the corpus striatum and extends into the frontal and olfactory lobes. The descending cornu curves ventrally into the temporal lobe, where it ends in that portion of the hippocampal gyrus known as the uncus. The extensive rounded

elevation in the floor of the descending cornu is the hippocampus.

Forming a part of the medial wall of each ventricle is the body of the fornix, the two halves of which join in the midline (cf. median sagittal section) to form a triangular mass. Anteriorly the fornix takes the form of two columns of fibers which curve ventrally and disappear (since they pass into the thick mass which forms the floor of the cavity, and then curve posteriorly to reach the mammillary bodies, from which they pass dorsally to reach the optic thalami in the lateral walls of the diencephalon). Posterior to the body of the fornix, its two crura diverge rapidly, and each forms the thin border, or fimbria, of the corresponding hippocampus. This may be disclosed upon one side by removal of the chorioid plexus which supplies the interior of the lateral ventricles, entering along the line between the corpus striatum and the fimbria where there is only a thin epithelial lamina. Draw the brain thus dissected to show the floor of the lateral ventricles, dorsal view.

By gently detaching the ventral ends of the temporal lobes, the hippocampus and the adjacent remaining portions of the temporal and occipital lobes may be lifted in one piece and turned forward, displaying beneath them the dorsal surface of the diencephalon and the mesencephalon which this portion of the cerebral hemisphere overlaps. Note that in the middle of the diencephalon anterior to the pineal body there may be seen the chorioid plexus which forms the thin roof of the third ventricle. By removing this the narrow slit of the third ventricle is disclosed from above. between the thick walls formed by the optic thalami. If the reflected portion of the cerebral hemispheres be pulled well forward, the Y-shaped foramen interventriculare will now be seen beneath the body of the fornix connecting the lateral ventricles with the third ventricle. Cut through the body of the fornix and thus remove entirely the reflected portion of the cerebral hemispheres.

## 3. Dissection of the Cerebellum.

By a series of horizontal slices, remove the dorsal portion of the cerebellum until the level of the entrance, upon each side, of the various bundles of fibers from below into the cerebellum, is reached. Cut away the middle region or vermis of the cerebellum, which lies over the fourth ventricle, and carefully detach it, leaving in place as much as possible of the anterior and posterior medullary vela which form the actual roof of the cavity. Separate and identify the three bundles of fibers which enter the cerebellum upon each side as follows: (a) the brachium conjunctivum, which enters it from the dorsal portion of the peduncle of the cerebrum; (b) the brachium pontis which enters it from the pons; (c) the corpus restiforme which enters it from the lateral funiculus of the medulla.

#### 4. The Brain Stem.

The dissection of the cerebral hemispheres and the cerebellum, as above directed, leaves that portion of the brain which corresponds to practically the whole of the primitive vertebrate brain and is known as the brain stem. In the study of this, distinguish with care between those portions which are external surfaces, those which are interior surfaces of cavities, and those which are cut surfaces. Draw a dorsal view showing the parts identified; also, if time permits, a lateral view.

In the telencephalon note the corpora striata which lie in the floor of the lateral ventricles; the thickness of the lateral wall of the ventricle, the cut surface of which displays a gray cortical layer and a converging mass of white substance adjoining the corpus striatum. This white substance consists largely of the system of projection fibers known as the corona radiata, which extends from the cerebral cortex through the corpus striatum to enter the cerebral peduncles and thus reach the more posterior portions of the nervous system. By making an oblique longitudinal section through one of the corpora striata, the course of these fibers through it may be seen, forming the "inner capsule" of the organ, and giving it the peculiar appearance from which it has derived its name.

In the diencephalon note that the external surface of the optic thalami, which form its thick lateral walls, is exposed to view, and that between these rounded eminences the removal of the anterior chorioid plexus has disclosed anterior to the pineal body the slitlike cavity of the diencephalon, the third ventricle, from which the foramen interventriculare leads on each side laterally into the lateral ventricles. Note the line along the surface of each optic thalamus marking the attachment of the chorioid plexus (the tænia thalami). On the ventral and lateral surfaces of the thalamus note that the optic tract spreads out over the surface, a part of its fibers passing on to reach the geniculate body, which appears as a slight elevation in the angle between the thalamus and the midbrain, while still others enter the superior colliculus of the corpora quadrigemina.

In the mesencephalon note that the exposed surface is wholly external surface. The four eminences of the corpora quadrigemina are known as the colliculi, the two superior colliculi being larger and nearer the middorsal line, while the inferior colliculi are more laterally located. The trochlear nerves may now be plainly seen as they emerge from the dorsal surface of the brain posterior to the inferior colliculi. Note the large size of the cerebral peduncles which form the floor of the mesencephalon, and their connection anteriorly with the base of the cerebrum which they reach by passing medial to the optic thalami. Note their convergence posteriorly to disappear within the pons.

In the rhombencephalon note the thin roof of the rhomboid fossa or fourth ventricle. If this thin roof has been removed, the whole floor of the rhomboid fossa is exposed to view, bordered laterally by the cut surfaces of the bundles of fibers which enter the cerebellum, and by the lateral funiculi of the medulla. Anteriorly the fossa closes in beneath the corpora quadrigemina to form the aquæductus cerebri, and posteriorly it closes in similarly to form the canalis centralis of the spinal cord. In its floor may be seen bordering the longitudinal sulcus numerous paired elevations which indicate the location of certain of the masses of gray substance or "nuclei" of the medulla. From one of these the bundles of fibers which make up the acoustic nerve may be seen to extend transversely beneath the epithelial lining of the fossa, to issue from the surface at the lateral margins.

# 5. Demonstration of Horizontal and Transverse Sections through Mammalian Brains (Human if Available).

Identify the structures which appear in the various sections, and endeavor especially to follow (a) the cavities through the various regions of the brain and (b) the main tracts or bundles of fibers, particularly those which make up the cerebral peduncles, and the various commissures. Draw and interpret as many as time permits.

#### XII. THE CRANIAL NERVES AND SPECIAL SENSE ORGANS

Material.—The head¹ of the calf or sheep from which the brain was removed; whole heads sawn sagittally for demonstration of general topography; one or two sheep (or calf) skulls sawn horizontally and sagittally, and disarticulated skulls. For comparison with human relationships, models and demonstration dissections may be used.

#### A. THE GENERAL TOPOGRAPHY OF THE HEAD.

One-half of the heads may now be sawn by a median sagittal section into halves. After thoroughly washing the sawn surfaces under running water study these sections for general topography, identifying the various bones, noting relation of nasal cavities, mouth, superior and inferior regions of the pharynx, œsophagus, larynx; nasal septum, hard and soft palates, tonsils between the pillars of the fauces, tongue, epiglottis, and glottis; openings of auditory (Eustachian) tubes; location and character of teeth. Cf. with similar sections of the entire head, in which the cranial cavity containing the brain is also included; also with models of the human head, median sagittal section. *Draw*, *labeling the parts identified*.

### B. THE FLOOR OF THE CRANIAL CAVITY.

Study the floor of the cranial cavity, first fitting together the two halves if the head has been sawn. Use for comparison the previous study of the features of the bony floor of the human cranial cavity (p. 44). Note that here the floor is covered by the dura mater, which does not conform completely to the bony floor of the cavity. This is particularly true in the region of the sella turcica, where a fold of the dura forms a false floor, the diaphragma sellæ, beneath which the hypophysis, is located. The hollow stalk of the hypophysis, severed in the removal of the brain, may

<sup>&</sup>lt;sup>1</sup> This, after two or three weeks in formalin, should be washed for two or three days in running water to remove the formalin, and may be kept in water during the progress of the work in the intervals when it is not being used.

be seen anterior to the edge of the diaphragma sellæ, and upon either side of this stalk the cut ends of the carotid arteries appear. Note the relation of the folds of the tentorium to the lateral boundaries of the diaphragma sellæ.

By reference to the previous study of the ventral surface of the brain, identify the cut ends of the cranial nerves which pass through the dura mater to reach their respective foramina of exit from the cranial cavity. Draw this view of the floor of the cavity, showing these features.

Make sure of the identification of the optic, oculomotor, trochlear, abducent, and ophthalmic branches of the trigeminal nerve.

With a very sharp scalpel or curved seeker carefully cut through the dura mater to trace each nerve forward to its place of exit from the cranial cavity into the orbital fossa. The optic nerve takes a very short course to reach the optic foramen. The trochlear nerve takes a very oblique course through the dura along the medial surface of the tentorial fold, which must therefore be dissected away from the nerve with great care not to cut or break the nerve itself. The abducent nerve passes through the extensive cavernous sinus which lies in the sella turcica lateral to the hypophysis, so that to follow the nerve through this sinus involves the removal of the spongy structure and clotted blood which fill it. The ganglion of the trigeminal nerve (the semi-lunar or Gasserian ganglion) lies beneath the fold of the tentorium lateral to the cavernous sinus. Follow the ophthalmic branches of this nerve forward to the place where, in company with the trochlear, oculomotor, and abducent, they pass through the superior orbital fissure into the orbital fossa. With these nerves traced, the removal of the remaining portion of the dura mater may be accomplished without injury to the various nerve roots all of which should be left intact. Draw the bony floor of the cavity with the dura removed, showing the relation of the various cranial nerves to their foramina of exit.

# C. THE EYE AND THE NERVE DISTRIBUTION TO IT. (Cf. model of human eye.)

Taking care not to injure or lose the identity of the nerves which have been traced to their entrance into the orbital fossa, chip away the roof of this fossa by means of bone forceps, and thus disclose the eyeball and its surroundings in situ. Keeping each nerve intact, remove bit by bit the packing of areolar tissue and fat until finally the various muscles and their innervation are correctly and conclusively demonstrated, and their identification made certain by reference to the accompanying tabulation.

Record this dissection of the eyeball surroundings and innervation by a series of drawings.

Early in this dissection, the large lacrimal (tear) gland will be noted between the eyeball and the outer dorsal region of the rim of the orbit. Incidentally, also, note the course of the superior and inferior ophthalmic branches of the trigeminal nerve, as they cross the medial and lateral regions of the orbit respectively, each giving off small branches to supply local regions. Skillful dissection will disclose, also, the ciliary ganglion (sympathetic) located lateral to the optic nerve. Note connections between this ganglion and the oculomotor nerve, and trace numerous small nerves from the ganglion to the eyeball. As the dissection continues, after the trochlear, abducent, and superior branch of the oculomotor nerve have been traced to the muscles which they innervate, and the relationships thus worked out have been recorded, these muscles, together with the optic nerve, may be severed a short distance from their insertion into the eyeball, and the eyeball thus in part set free may then be turned forward thus giving access to the muscles which are more deeply located and are innervated by the inferior branch of the oculomotor. Retain all of the nerve and muscle connections which are worked out so that after the dissection is completed, all of the relationships may be clearly demonstrated.

The Eyeball (if possible fresh material should be used).—Note that in removing the eyeball the thin layer of skin, the conjunctiva, which covers the front, or exposed surface, of the eyeball, has been cut leaving intact, probably, the thin, cartilage-supported, semilunar fold (third eyelid or nictitating membrane) which crosses obliquely the medial region of the eyeball and has connected with it a firm mass, the Harderian gland. These structures may be used, together with the lacrimal gland and the openings of its ductules under the lateral region of the upper

Name of muscles in probable order of identification	Location	Origin	Insertion	Innervation
I. Levator palpebræ Dorsal to eyeball.	Dorsal to eyeball.	Posterior region of orbital Upper eyelid, wall.	Upper cyclid,	Oculomotor, superior branch.
2. Rectus superior	Dorsal to eyeball.	Posterior region of orbital wall.	Eyeball, anterior region, dorsal surface.	Oculomotor, s u p e r i o r branch.
3. Obliquus superior.	Dorso-medial to eyeball, and bending at right angles before reaching its insertion.	Dorso-medial to eyeball. Posterior region of orbital Byeball, anterior region, Trochlear, and bending at right wall.  Intero-dorsal surface.  its insertion.	Eyeball, anterior region, latero-dorsal surface.	Trochlear.
4. Rectus medialis.	Medial to eyeball.	Posterior region of orbital Eyeball, anterior region, Oculomotor, wall.	Eyeball, anterior region, medial surface.	Oculomotor, inferior branch.
5. Rectus lateralis Lateral to eyeball.	Cateral to eyeball.	Posterior region of orbital Eyeball, anterior region, Abducent.	Eyeball, anterior region, lateral surface.	Abducent.
6. Retractor bulbi (not present in man)	Surrounding the posterior half of the eyeball.	Posterior region of orbital Eyeball, around the mid-Abducent.	Eyeball, around the middle region.	Abducent.
7. Rectus inferior	Ventral to eyeball.	Posterior region of orbital wall.	Byeball, anterior region. Oculomotor, inferior ventral surface.	Oculomotor, in ferior branch.
8. Obliquus inferior	Ventral to eyeball, and parallel to the rim of the orbit.	Medial wall of the orbit.	Eyeball, anterior region, latero-ventral surface.	Eyeball, anterior region, Oculomotor, in ferior latero-ventral surface.

eyelid, to determine whether the specimen is from the right or the left side. Note form and size of the eyeball, the point of attachment of the optic nerve, and places of insertion of the various muscles. Remove these together with the associated fat and connective tissue. Note that the outer wall of the eye ball consists of a thick, tough, whitish, skeletal structure, the sclera, except in the region where the transparent cornea, resembling in life a watch crystal, takes its place on the exposed surface. Through the cornea the colored iris with a circular opening in the middle, the pupil, may be seen (cf. living eye), and through the pupil the crystalline lens, also perfectly transparent in life, but rendered opaque by preserving fluids.

With the specimen under water carefully cut the wall of the eyeball along an equator which separates posterior from anterior halves, and thus open into the posterior chamber back of the lens. Note the somewhat jelly-like vitreous humor filling this cavity, and the delicate retina lining it (frequently detached in the process of dissection); the latter, composed of nerve tissue, is the receptive organ for light stimuli (cf. microscopic study, pp. 63, 64). Note the "blind spot" where the optic nerve enters. Outside of the retina, between it and the sclera, is the deeply pigmented chorioid layer.

In the anterior half of the eyeball note the extent of the retina. The crystalline lens will be seen supported by a suspensory capsule from the circle of ciliary processes borne on the ciliary fold which lies between the chorioid and the outer circumference of the iris. Draw an internal view of (1) the posterior and (2) the anterior half.

The small anterior chamber, filled with a watery fluid, the aqueous humor, lies in front of the lens and may be opened into from behind by carefully removing the lens and its capsule. This exposes the ciliary fold and the inner surface of the iris, in which there are both radiating and circular muscle fibers. Draw the interior of the anterior half with the lens removed.

External Study of the Living Eye and Its Surroundings in the Human Subject.—Note eyebrows, eyelids supported by tarsal cartilages and fringed with eyelashes; the thin skin (conjunctiva) which lines the eyelids and covers the front (exposed) surface of the eyeball; by turning back the eyelid, the outlines of the yellow tarsal glands, connected with the roots of the eyelashes, may be seen; the moist condition of the conjunctiva is due to the lacrimal secretion which reaches the surface of the conjunctiva through orifices of ductules above the lateral angle and is drained off through the orifices of the naso-lacrimal duct, which are located on the lacrimal papillæ upon the eyelids above and below the medial angle. In this region also, look for the semilunar fold of conjunctiva (the nictitating membrane) and the lacrimal caruncle medial to it. Draw the eye with its surroundings as seen from the front.

Examine the various optical models of the eye which demonstrate the method of formation of the image of an object upon the retina, and the range of accommodation for vision of objects at varying distances.

#### D. THE OLFACTORY ORGAN AND ITS ACCESSORIES.

For this work students may group themselves in pairs, each pair being supplied with (1) a whole head from which the brain has been removed, and (2) a head which has been sawn as above directed in a median sagittal plane. The nasal region of the first should be sawn in a series of parallel transverse sections at right angles to the long axis of the nose and about 11/2 inches apart. In so doing be careful not to injure the tongue. Wash the sawn surfaces under running water to remove debris. In the case of the half heads, if the nasal septum is still in place, remove it, carefully noting what parts are of connective tissue, and of cartilage, and identifying the bones which contribute to it. Make a careful study and comparison of these preparations, using each point of view as a means for identifying the structures which appear in the other one. Note the following points: extent and boundaries of the nasal cavities; nature of lining mucous membrane and devices for increasing its area; division of cavity into pars olfactoria, and pars respiratoria; the cribriform plate of the ethmoid, through the numerous fenestræ of which the fiber bundles of the olfactory nerves pass to reach the mucous membrane of the pars olfactoria of the nasal cavity; the conchæ, with their supporting bony framework of the scroll-like maxillo-turbinal, maso-turbinal, and ethmo-turbinal bones.

Draw transverse sections at various levels, showing the structures and relationships which have been worked out, and a medial view of the interior of the nasal cavity.

Dissect out the maxillary concha from the nasal cavity of the half head, leaving intact the surface of its anterior region upon which a small pore (the orifice of the naso-lacrimal duct) may be seen; cut away also the superficial layer of the nasal concha thereby laving open the sinus which it encloses; remove the freely projecting ends of the ethmoid conchæ, and note the complicated character of the sinuses and scrolls thus displayed. In the walls of the nasal cavity as thus exposed, look for orifices leading into the frontal, nasal, and maxillary sinuses. Pass probes of fine wire, or other suitably flexible material, as far as possible into all probable orifices of this kind, including that of the naso-lacrimal duct, and after recording the location of each of these upon a drawing of the lateral wall of the cavity, complete the identification of these orifices by dissecting away the bony walls sufficiently to follow the probe previously introduced until the relationships have been clearly determined. In the case of the naso-lacrimal duct note that the tube is one of considerable size which passes very obliquely through the outer plate of the maxillary bone and through the lacrimal foramen into the orbit of the eye. By means of dotted lines added to the drawing of the lateral wall of the cavity show the connections thus worked out between the nasal cavity and the various sinuses, and the course of the naso-lacrimal duct.

## E. THE EAR. (Cf. model of the human ear.)

The internal ear, the real auditory organ, lies within the thickened or petrous portion of the temporal bone and consists of a thin-walled, complicated structure known as the membranous labyrinth, enclosed within a thin layer of bone known as the bony labyrinth. The membranous labyrinth is filled with a fluid known as the endolymph, and the space between the membranous and the bony labyrinth is filled with perilymph. Accessory to the internal ear, are the cavum tympani (middle ear) and the external ear, which afford a continuous channel through which the sound waves reach the internal ear.

The ear region may be reached by sawing horizontally through the head (or more conveniently the half head) just above the level of the internal acoustic meatus, the external acoustic meatus, and the pharyngeal orifice of the auditory (Eustachian) tube.

The external ear consists of the auricle (usually removed in skinning the specimens) and the acoustic canal, which leads through the external acoustic meatus to the cavity of the middle ear, the cavum tympani, from which it is separated by the delicate membrane of the tympanum stretched across its inner end. By dissecting away the soft parts and carefully chipping off the bony roof of the acoustic canal and the cavum tympani, these cavities may be opened from above with the membrane of the tympanum in place between them.

The cavum tympani communicates with the pharynx through the auditory or Eustachian tube which may also be laid open from above. (There also open into the cavum tympani from below, the large cavities or "cells" of the mastoid bone, as may be demonstrated from a preparation of the human temporal bone sawn through the cavum tympani and the mastoid process.)

The membrane of the tympanum bears attached to its inner surface, the first of a chain of three tiny bones, the auditory ossicles; this first ossicle is the malleus, and is in turn articulated with the incus, while the latter bears the stapes; the stapes has a ring-shaped portion which fits into the fenestra vestibuli, an opening in the bony wall of the vestibule of the internal ear. Thus sound vibrations which move the tympanic membrane are communicated through this chain of bones to the perilymph and through this to the endolymph, and so stimulate the receptive organs which are in the lining of the membranous labyrinth. A second fenestra in the bony wall of the labyrinth, the fenestra of the cochlea, also opens into the cavum tympani beneath the fenestra vestibuli, and the membrane which covers it furnishes the amount of movability in the wall of the perilymph cavity necessary to permit the vibrations of the fluids.

Two muscles may be seen in the cavum tympani, (1) the tensor tympani, which arises from the medial surface of the wall of the auditory tube and is inserted, by means of a tendon which is bent nearly at right angles, into the malleus, and (2) the stapedius

muscle, which arises from the posterior wall of the tympanum and is inserted into the stapes.

The finer details of the internal ear cannot be made out from such material as this, but much of the general topography can be determined. Starting with the internal acoustic meatus, note that both the acoustic and facial nerve enter the petrous portion of the bone. By chipping away the surface of the bone with the bone forceps follow the course of the facial nerve along the line bounding middle and internal ear, and note that the acoustic nerve, which is more deeply located, has a ganglion within the acoustic meatus, beyond which point its two divisions diverge, the cochlearis passing forward and the vestibularis downward and backward. By further chipping away the bone certain portions of the labyrinth itself may be located.

The labyrinth consists of a vestibule, which contains two distended portions of the membranous labyrinth known as the saccule and the utricle, connected with each other through the endolymphatic duct. From the vestibule leads (1) the very elaborate coiled structure, known as the cochlea, located ventral and anterior to it, and (2) the three delicate semicircular ducts, located dorsal and posterior to it (cf. dissection of the dogfish showing these canals). The membranous portion of the cochlea connects with the cavity of the saccule through a slender duct known as the ductus reuniens. Within the lining of the vestibule and the cochlea are the receptive organs of the sense of hearing, the spiral organ of Corti (cf. p. 65), while the semicircular canals are the organs of equilibration.

Show by diagram the relation of the parts of the ear.

# F. THE FLOOR OF THE MOUTH, AND THE TONGUE.

With the aid of a saw remove from those specimens of sheep's heads which were not sawn sagittally, all the remaining portion dorsal to the surface of the tongue, and study the tongue and floor of the mouth. Use for comparison, the previous study of the median sagittal section of the head, also the interior of your own mouth, viewed with a mirror, or the interior of the mouth of a fellow-student. Compare, further, with demonstration dissections and models of the human tongue and larynx.

Floor of Mouth.—Note the kind and arrangement of teeth, the grinders (molars) in the posterior region and the cutters (incisors) in front, separated from the molars by a toothless interval. Note in connection with this, the complete absence of incisors in the upper jaw.

Note the tall papillæ, filiform in shape, of the tactile variety in the lining of the cheeks, and beneath the tongue.

The Tongue.—Note its shape and extent. It is a muscular organ capable of taking a great variety of forms (cf. movements of your own tongue). The whole upper surface is thickly covered by papillæ, the majority of which are minute and of the filiform variety, tactile in function. Among these are distributed the true taste papillæ of the fungiform type, small in the distal region of the tongue but very large in the proximal. Along each side of the tongue in the proximal half, find a double row of large papillæ of a third type, the circumvallate, consisting of an elevated circular center, with a furrow surrounding it and a circular ridge surrounding the whole. Connected with the posterior part of the tongue, note the presence and position of the epiglottis, which guards the glottis, the orifice of the larynx. Locate by palpation the hyoid bone which forms the skeletal support of the tongue; by dissection demonstrate the median part, or body, of the hyoid and two pair of cornua, and note that the lesser cornua are attached to the skull by a series of ligaments, and cartilages or bones (cf. the styloid processes of the human skull), while the greater are attached by ligaments to the thyreoid cartilage of the larynx (cf. dissection of the latter, pp. 98-100).

Draw a view of the floor of the mouth and the tongue.

# XIII. THE THORACIC VISCERA (PLUCKS) OF SOME LARGE MAMMAL

Material.—Thoracic viscera (lamb or pig) obtained fresh from the abattoir, with as little mutilation as possible. The whole length of trachea, and the larynx and tongue should be included, also, at the posterior end, a portion of the diaphragm (and the liver in the case of one or two specimens).

Orientation and Identification of the Structures Present.—Although the liver is an abdominal rather than a thoracic organ, its presence in at least a single specimen will help in orienting the other organs, and incidentally the general form and appearance of the liver itself should be noted. Identify its various lobes, the gall bladder, and the cystic duct leading from it to the duodenum (the latter not present here). Cut off and preserve small portions (an inch or less in each dimension) of the liver in 70% alcohol or 5% formalin for later study. In case the material is absolutely fresh, smaller portions may be preserved for imbedding and sectioning (see p. 22).

Note that the outer surface of the lung is covered by the thin smooth visceral pleura, which is continuous with both the dorsal and the ventral mediastinal pleuræ. Note that the heart, enclosed within the pericardium, lies (together with the large blood vessels which are connected with it) in the ventral mediastinal space, and that the esophagus and the dorsal aorta are in the dorsal mediastinal space. If the material is sufficiently complete, trace these, and also the posterior vena cava, through the diaphragm. Sever the trachea and the esophagus an inch or two below the larynx. Dissect the esophagus well away from the dorsal wall of the larynx, leaving it attached only at the anterior end where it joins the pharynx. Cut off and discard the distal half of the tongue, together with any hanging, ragged ends of muscles and fat, and preserve the larynx for later study in 5% formalin.

### A. THE TRACHEA, BRONCHI, AND LUNGS.1

Inflate the lungs by means of a large blowpipe inserted through the trachea and guarded by a compressible rubber tube as a mouthpiece, which may be pinched together to prevent air returning from the lungs to your own respiratory passages.

Note that each lung has a large posterior lobe and a smaller bilobed anterior one and that the right lung has an additional lobe which fits into the space posterior to the heart.

With the dorsal surface of the material uppermost, expose the trachea by removing the œsophagus and turning the aorta forward, start at the anterior end and follow the trachea until it divides into the bronchi which enter the various lobes of the lung. By removing the lymph glands, fat, and areolar tissue from the surface of the bronchi and the pulmonary veins and arteries which accompany them into the various lobes of the lungs, the whole plan of the air and blood supply to the lungs may be worked out. The blood vessels should also be traced in the opposite direction to demonstrate their relation to the heart. Note that the pulmonary veins of the various lobes enter the left auricle of the heart, the thin dorsal wall of which is immediately ventral to the large bronchi. Show by diagram the whole plan of air and blood supply to the lungs.

In a single posterior lobe trace out by teasing and tearing the soft tissues, the entire course of the bronchus and its branches, together with the course of the corresponding veins and arteries. Note that the arteries are anterior and lateral to the corresponding bronchial tubes, while the veins are medial and posterior to them. Draw this dissection.

Note that the smaller bronchial tubes, as well as the bronchi and trachea, contain in their walls incomplete rings of cartilage, which, while insuring a constantly open condition of the lumen, allow at the same time a considerable range of variation in caliber, which complete rings would prevent. In the smaller tubes the rings become very irregular, and the ultimate branches, the bronchioles, possess no cartilage. These, like the alveoli into which they lead, are too minute for demonstration by gross dissection.

<sup>&</sup>lt;sup>1</sup> Keep the material in cold storage (or on ice) in wet wrappings at least until the study of the lungs is completed.

Note that a fragment of lung tissue from which the air has been driven by pressure between the fingers, still contains so much air in its alveoli that it will float when placed in water. Finally detach the lungs and trachea from the heart and its large blood vessels by severing carefully, one by one, the pulmonary veins and arteries at a point as far distant from the heart as possible. Discard the lungs and preserve the heart for later study, either in cold storage or in 5% formalin.

Examine with the microscope (Dem. Sl. Coll.) cross sections of the trachea showing the cartilaginous rings of the walls and the ciliated epithelial lining; and sections through the lung showing the alveoli inflated, and their relation to the bronchioles which lead into them. Draw whatever you are able to identify in the way of details from these sections.

# B. THE LARYNX. (Cf. compare with dissections and models of the human larynx.)

Note that the larynx is a differentiation of the anterior region of the trachea. In the walls of the larynx locate, by palpation, the broad thyreoid cartilage on the ventral and lateral sides, the signet-ring shaped cricoid cartilage with its narrow portion posterior to the thyreoid and its broad portion dorsal to it, and the pair of arytænoids which project anteriorly, one upon each side, between the lateral borders of the thyreoid and the broad dorsal region of the cricoid.

Note that the hyoid bone lies immediately anterior to the thyreoid cartilage, and serves as attachment for numerous muscles which are not associated with the larynx; the cut ends of these muscles are here seen, and should be carefully removed so as to expose the external surface of the hyoid bone, the relation of which to the thyreoid cartilage will then be made clear. In this dissection preserve, if possible, the chain of ligaments and cartilages which serves to attach each of the lesser cornua of the hyoid to the skull, thus suspending the tongue and the larynx from the skull (see p. 95).

By the usual method of dissection of muscles work out the musculature of the external (ventral and lateral) surface of the larynx, identifying (1) the pair of thyreohyoid muscles which lie

one upon each side of the prominent median process ("Adam's apple") of the thyreoid, with the origin and insertion which is indicated by their name; (2) the posterior end of each of the cricothyreoid muscles extending from the posterior edge of the thyreoid cartilage to the ventral region of the cricoid; (3) the insertions into the lateral surfaces of the thyreoid, of the inferior constrictor of the pharynx, which encircles the anterior end of the esophagus and holds it firmly in place; (4) portions of the sternothyreoid muscles which have their insertion into the posterior border of the thyreoid cartilage. Draw either the ventral or lateral view of the larynx and hyoid bone showing this musculature.

Remove the œsophagus from its attachment to the dorsal wall of the larvnx by severing the constrictor of the pharynx upon each side. Cut transversely across the belly of the thyreohyoid muscle upon one side and reflect the two ends of the muscle. Make a clean longitudinal cut through the whole length of the thyreoid cartilage a little to one side of the midventral line, and carefully lift the anterior end of the piece thus separated and free it from its loose attachment to underlying parts taking care not to destroy the crico-thyreoid muscle, the extensive origin of which from the inner surface of the thyreoid will now be fully demonstrated. With this muscle intact, the detached portion of the thyreoid may now be completely reflected, thus making possible the dissection of the deeper muscles of the larynx, which should be identified from their location and attachment as follows: (1) the dorsal crico-arytænoid or crico-arytænoideus posterior, (2) the lateral crico-arytænoid, (3) the thyreo-arytænoid, and, (4) the arytænoid muscles, transverse and oblique, vestiges of which may be seen stretching between the anterior ends of the arytenoid cartilages. Note, incidentally, that slender muscles pass forward from the laryngeal cartilages to the epiglottis. Draw a lateral view of this dissection.

Lay open the larynx by a median incision through its whole length along the middorsal line. Note that the vocal folds of the mucous membrane, with their underlying elastic ligaments, stretch from their ventral attachment to the inner surface along the midline of the thyreoid, to the vocal processes of the arytenoids, which lie beneath the mucous membrane on either side of

the dorsal midline. Above each fold is an elongated depression, the ventricle, which is bounded anteriorly by the ventricular fold. Note that while the vocal folds are approximated and stretched into the position for vocalization of the breath by the contraction of the cricothyreoid muscles which pull forward upon the thyreoid cartilage, the tension of the vocal folds is regulated by the various combinations of contractions of the other muscles of the larynx, which are inserted, as shown by the above dissection, into the muscular processes of the arytænoids. *Draw the interior view of the larynx*.

### C. THE HEART AND LARGE BLOOD VESSELS.

Remove the loose pericardial sac which surrounds the heart, noting that a reflected portion of the pericardium fits tightly to the heart and forms its outer layer. By palpation and by external examination locate the four chambers of the heart (i.e., right auricle and ventricle, and left auricle and ventricle), and identify the blood vessels connected with each. Draw dorsal and ventral views.

Make a cross section near the apex of the heart to demonstrate the circular form of the cavity of the thick-walled left ventricle, and the crescentic form of the cavity of the thin-walled right ventricle. Note the tendinous attachments of the tricuspid and bicuspid (mitral) valves in the right and left ventricles, respectively. By probing, show that the pulmonary artery leads from the right ventricle and the aorta from the left.

Taking care to avoid the anterior and posterior venæ cavæ which open into the right auricle, make a longitudinal slit through the wall of both the right auricle and right ventricle, thus laying open the two cavities. Note the thin, distensible walls of the auricle, the place of entrance of the veins, the auriculo-ventricular orifice through which the blood leaves the auricle and enters the ventricle and the tricuspid valve which guards this orifice. *Draw*.

Make a similar dissection of the left side of the heart, noting the entrance of the pulmonary veins into the auricle, and the bicuspid valve guarding the auriculo-ventricular orifice.

Make an incision transversely through the pulmonary artery about an inch above the heart and look down toward the heart to see the three semilunar valves which guard the artery. Lay the artery open by a longitudinal slit passing into the ventricle and study the valves more carefully. *Draw*.

Similarly dissect and study the semilunar valves of the aorta. Draw a diagram showing the physiological anatomy of the heart and large blood vessels, and indicate by arrows the course of the blood through them.

### D. PHYSIOLOGICAL DEMONSTRATIONS.

### 1. The Contraction of the Heart in the Frog.

The animal used for this purpose should be either pithed or etherized. Note that a complete cardiac cycle consists of (1) a wave of contraction (systole) which proceeds from auricle to ventricle and drives the blood on, emptying each part successively, and (2) a period of relaxation (diastole), during which the auricles refill from the veins, and the ventricle (the frog heart has only one) refills from the auricle.

### 2. The Sounds of the Beating Heart in the Living Human Subject.

By means of the phonendoscope (or some other form of stethoscope) or by placing the ear directly in contact with the chest over the region of the heart, listen carefully to the sounds and learn to distinguish them. The first sound is due to the ventricular systole and is attributable to a combination of causes among which the vibration of the contracting muscle is undoubtedly the chief one. The second sound, which is the shorter and the sharper one, is caused by the sudden and simultaneous closure of the semilunar valves which guard the entrances to the pulmonary artery and the aorta, respectively. Determine the rate of heart beat when the subject is sitting, standing, and after vigorous exercise *Record*.

### XIV. THE BLOOD VESSELS

### A. GROSS ANATOMY OF THE ARTERIES AND VEINS.

Preparation of Material (Cat, Rabbit, White Rat, or Guinea Pig).—The study of the circulatory system of an animal is greatly facilitated by filling and slightly distending the blood vessels, previous to dissection, with some fluid which will solidify within the vessels and which, being brightly colored, will enable the student to follow the course of the various vessels with ease. Such a fluid injection mass may, moreover, be distinctively colored to differentiate the various parts of the circulatory system.

There are many different injection masses used, the chief requisite in preparation for gross dissection being (1) that the liquid should flow freely through the small tubes or cannulas used to introduce it into the various vessels; (2) that the fluid should contain suspended particles too large to pass through the capillary vessels into the tissues; (3) that the coloring matter should not be soluble, as it would then not remain confined to the blood vessels, but would stain the surrounding tissues; (4) that the mass should solidify within the vessels, but not too rapidly.

The following **injection mass**<sup>1</sup> fills the above requirements satisfactorily. Make a gelatine solution of one part gelatine to seven or eight parts (by weight) of water. Soak the sheets of gelatine in the cold water and gradually heat it until the gelatine is dissolved, taking care not to burn it. To one volume of dry corn starch (mixed with an amount of powdered dry pigment sufficient to give the requisite depth of color) add three or four volumes of the warm (but not boiling) gelatin solution. Mix thoroughly and strain, while the mixture is still warm, through a fine wire strainer into a small clean jar, which, to be used conveniently, should be wide mouthed and should not be filled more than half full. The mixture thus made may be kept for several days by the

<sup>&</sup>lt;sup>1</sup> Cf. Rand, The Skate for Classes in Comparative Anatomy; injection methods. American Naturalist, Vol. XXXIX, p. 365, 1905.

addition as soon as it has solidified, of a sufficient quantity of some coal-tar disinfectant to cover the top with a thin layer.

When the mass is to be used, first wash the disinfectant from the surface by means of cold water, then let the jar stand in a pan of hot water (but never boiling, since that would cook the starch) for a few minutes to melt the gelatine. Keep the water in the pan hot by changing it from time to time as the work proceeds. Lay in the same hot water the implements to be used, such as glass syringes, several glass cannulas made from glass tubing drawn out into a tapering nozzle for insertion into blood vessels, short lengths of rubber tubes for connecting cannulas with syringes, and a sponge, or mass of absorbent cotton. Artery clamps for the temporary closing of a cut vessel to prevent loss of the injection mass when the syringe is withdrawn, are very convenient, though little plugs of cotton or the simple pressure of the finger may be used frequently for the same purpose.

In injecting, fill the syringe with the thoroughly stirred injection mass and then attach the rubber tube, into the end of which the glass cannula has been inserted.

In case the injection is to be made through a small blood vessel it will be necessary to insert the cannula into a slit in the wall of the vessel before attaching it to the syringe, since, owing to the collapsible nature of small blood vessels, particularly veins, the most effective method of inserting the cannula is by blowing the edges of the slit open using the cannula itself with the rubber attached as a blowpipe, and thrusting the point of the cannula in as the slit opens up.

In case the injection is made directly into the heart or some large vessel, the attachment to the syringe may be made, and the cannula itself filled with the injection mass, before it is inserted.

In any case, after the insertion of the cannula is made and the syringe is attached, the injection mass should be driven in with a slow steady pressure of the piston, while the cannula is firmly held in place with the thumb and finger. Never force the injection against a decided resistance but if you have reason to believe that the injection is not complete, halt the process and seek for the source of the resistance. At times, the squeezing of hot water from a sponge over the region which is being injected, facilitates

the process, since if the process is unduly prolonged the injection mass will harden either in the cannula or the blood vessels. Moreover, a previous injection may at any time be supplemented by an application of warm water to melt it, and then injecting more of the mass at any desired point. When an injection is completed, the cannula is withdrawn, the escape of the mass prevented by a ligature around the vessel, or by an artery clamp, or simply by pressure from the fingers or a wad of wet cotton, while the specimen is held under a stream of cold water until the injection mass is hardened.

To prepare the specimen for injection, lay back the skin from the ventral surface of the neck, trunk, and proximal region of the legs; open into the thoracic cavity by a longitudinal incision through the series of costal cartilages on each side of the sternum. Tie a ligature tightly around the sternum near its anterior end to prevent the escape of blood (and later of the injection mass) from the cut ends of the sternal veins and arteries. Sever the sternum transversely posterior to the ligature and, after freeing the posterior end from the diaphragm, remove it together with the stumps of the costal cartilages and thus expose the heart within the pericardium, in situ in the thoracic cavity. Carefully slit open the pericardium. From your previous study of the organ its parts may be readily identified.

Open the abdominal cavity by a longitudinal incision a little to one side of the midline. Plug the rectum with cotton inserted through the anal opening, to prevent the escape of fæcal matter (especially necessary in case the animal used is a cat).

Identify by reference to the list given below, the various blood vessels through which the injections are to be made, and, where indicated, pass a ligature of coarse thread or fine cotton string about the vessel and tie the ends loosely in a single knot, laying them carefully out, one on each side so that they may be conveniently drawn up tightly before the cannula is finally removed.

# r. Systemic Veins and Pulmonary Arteries with Blue.

In case the animal is as large as an adult cat or rabbit, this injection may conveniently be made through the femoral vein which may be readily exposed upon either side in the angle between

the leg and the body, where it lies superficially beside the somewhat smaller femoral artery. A ligature should be passed around the vein and its accompanying artery separately before the injection is begun. Insert the cannula by the method above described (p. 103) through an incision in the wall of the vein distal to the ligature, and inject toward the heart, *i.e.*, in the direction of normal blood flow. The injection mass will thus fill the veins along the whole course from the point of injection to the heart and will, moreover, back into the other branches of the posterior vena cava and into the anterior vena cava until it meets the first valves guarding these. It will also go forward through the heart and fill the pulmonary artery and its branches. If the injection is not successful, the other femoral artery may be tried, or in the case of a small animal the injection may be made much more readily through one of the jugular veins in the neck region.

### 2. Systemic Arteries and Pulmonary Veins with Red.

Here again unless the animal is too small, the injection may be made from the femoral region through the femoral artery, and will, if successful, fill not only the whole arterial system (as will be seen by watching the progress of the injection mass in the numerous small arteries of the mesentery), but will force its way past the bicuspid valve of the heart and back into the pulmonary veins.

In case the animal is too small for ready injection through the femoral artery, inject through the left ventricle directly through a puncture made through the thick ventricular wall by means of the cannula. If a plug of cotton is inserted as the cannula is withdrawn, the puncture may be successfully closed, though the elasticity of the thick muscular wall itself will usually accomplish this closure.

# 3. Hepatic Portal System with Yellow.

This injection may be made through any convenient mesenteric vein. If one place fails, clamp it and try another. Close by clamp or by temporary pressure of a wad of wet absorbent cotton, or by the finger.

After injecting, cool the specimen under the cold water faucet, to harden the gelatine. Make a slit in the wall of the stomach and

remove the contents under a stream of water. In case the animal is a rodent, e.g., rabbit, white rat, or guinea pig, ligate the large intestine near the entrance into it of the cæcum, make an incision at each end of the cæcum and, after introducing a tube from a cold-water faucet into one of the incisions, turn on the water gently to wash out the contents of the cæcum. In case the animal used is a cat, the large intestine should be emptied by a similar method. This process, though somewhat unpleasant, obviates greater inconvenience later. The specimen may be kept on ice in moist wrappings, or in running cold water for two or three days, but if it is desired to work on it longer, it should be skinned and preserved in 5% formalin, followed as usual by thorough washing in water before using.

Directions for Dissection.—In general, dissection consists in removing the connective tissue which surrounds the blood vessels and holds them to adjoining structures, and by this means following out the vessel and its branches to the organs or regions which they supply. As the main veins and arteries follow the same general course, it will often be necessary to follow them out in a given region simultaneously. In no case should a blood vessel be cut until its connections have been fully determined and recorded.

The record of the dissection must be kept in the form of a diagram (or a series of diagrams) which may either combine both the venous and arterial systems, or, if showing the two systems separately, should indicate correctly their anatomical relationships. The direction of blood flow should be indicated by arrows, and colors (corresponding to those used in injecting) may be used to distinguish the different systems.

The most convenient order to take in dissecting is as follows:

- 1. The anterior vena cava<sup>1</sup> and its branches, working anteriorly from the heart, first very carefully laying open the narrow anterior end of the thoracic cavity by cutting through the attachments of the first pair of ribs to the sternum.
- 2. The anterior branches of the aorta which issue from the thoracic cavity just dorsal to the anterior vena cava.

 $<sup>^{1}</sup>$  In certain primitive mammals, e.g., the rabbit, there are two anterior venæ cavæ, a right and a left.

- 3. The blood vessels of the thoracic cavity which comprise in addition to branches of the anterior vena cava and the aorta, the entire pulmonary system of arteries (injected blue) and veins (injected red).
  - 4. The hepatic portal system and the mesenteric arteries.
- 5. The branches of the posterior vena cava and the remaining branches of the aorta.

List of the principal blood vessels to be identified:

### 1. Systemic System.

(a) Arteries (injected red).

Aorta, leading from left ventricle and forming an arch from which the anterior branches are given off.

i. Anterior branches:

Brachiocephalic.

Right subclavian.

Right carotid.

Left carotid. (This, in some species, e.g., cat, guinea pig, is a branch of the brachiocephalic.) Left subclavian.

ii. Thoracic branches:

Intercostals (several pairs).

iii. Abdominal branches:

Phrenic.

Cœliac axis.

Hepatic.

Coronary (i.e., to the stomach).

Splenic.

Superior mesenteric (in some species, e.g., guinea pig, this is united with the cœliac axis).

Suprarenals (paired).

Renals (paired).

Spermatic or ovarian (paired).

Inferior mesenteric.

Lumbar (in segmentally arranged pairs).

Iliolumbars.

Common iliacs (in some species, e.g., cat, there is no common iliac, the external and internal iliacs being given off from the aorta as separate branches).

External iliac, giving off branches to external pelvic region and continuing as the femoral artery into the leg.

Internal iliacs, passing into the pelvic cavity where branches are given off to the bladder and other pelvic organs, and to the pelvic walls and the gluteal region (to trace these into the pelvic cavity the pubic bones must be separated by cutting through the symphysis).

Caudal (median continuation of the aorta).

### (b) Veins (injected blue).

i. Anterior branches, entering anterior vena cava:

Brachiocephalics, right and left (in case there are two anterior venæ cavæ, e.g., rabbit, there are no brachiocephalics):

Subclavian.

External jugular.

Internal jugular.

### ii. Thoracic branches:

Right azygos, receiving intercostal branches, and entering the anterior vena cava.

iii. Abdominal branches, entering posterior vena cava:

Phrenics.

Hepatics.

Renals.

Spermatics or ovarian (note lack of symmetry in right and left sides).

Iliolumbars.

Common iliacs, with divisions and branches corresponding to those of the iliac arteries.

# 2. Portal System (injected yellow).

This consists of branches from the digestive tract, pancreas, and spleen which unite to form the hepatic portal vein. This enters the liver and divides, distributing the blood to its capillaries.

### 3. Pulmonary System.

(a) Arteries (injected blue).

Pulmonary artery, leading from the right ventricle, its right and left branches distributed to the corresponding lungs.

(b) Veins (injected red).

Pulmonary veins from the various lobes of the lungs into the left auricle.

#### B. THE CAPILLARIES.

For the study of capillaries, it is necessary to use either thick microscopic sections or small fragments of tissues in which the capillaries have been injected. Study with care and draw the details of a small region of one or more preparations, noting in each case the adaptation of the form of capillary network to the structure of the tissue in which it is located.

The following preparations are suggested:

- 1. Transverse section of intestine: Note the capillaries of the villi and of the muscular coats.
- 2. Section of tongue: Distinguish the capillary network of the various sets of muscles.
- 3. Section of lung tissue: Note that the capillaries of the alveoli form an almost continuous layer in the alveolar wall.
- 4. Surface mount of small pieces of injected mucous membrane of rodent colon, showing capillary network in relation to short tubular glands.

### C. STRUCTURE OF WALLS OF BLOOD VESSELS.

Study cross sections of veins and arteries and compare as to thickness of the walls and form of lumen. Note that in both there are three coats, the tunica intima, comprising the endothelial lining (supported, in the case of the artery, upon an elastic membrane), the tunica media, consisting of layers of elastic connective tissue and plain muscle fibers (this layer in the veins possesses less of the elastic and muscle tissue and is mainly of fibrous connective tissue), and the tunica adventitia, consisting mainly of elastic

and fibrous connective tissue. Draw, showing characteristic differences.

Examine sections through various organs for identification of veins and arteries which appear in such sections.

# D. LOCATION OF SUPERFICIAL BLOOD VESSELS IN THE LIVING HUMAN SUBJECT. (Cf. manikin and atlases or text-books for identification of vessels.)

**Arteries** (distinguished from veins by the presence of a pulse). Carotid, facial, temporal, axillary, radial, femoral.

Veins.—Jugular, axillary, femoral, popliteal, and in many regions of the body, particularly in the distal portions of the extremities, conspicuous meshworks of subcutaneous veins. Choose some region as, for example, the back of the hand or the flexor surface of the wrist, and carefully study this meshwork as to (1) symmetry of the two sides of the same individual, (2) amount of variation exhibited by different individuals, (3) the location of valves, readily demonstrated by exerting sufficient pressure between the region under observation and the heart to prevent the onward flow of blood, which thus distends the vein between the point where the pressure is exerted and the nearest valve, since the blood accumulates proximal to the latter and is prevented by it from flowing backward. Record by notes or by drawing.

# E. DEMONSTRATIONS OF THE MOVEMENT OF THE BLOOD.

- r. Demonstration of the circulation of blood in a three-day chick: Break the egg into a dish of warm physiological salt solution, being careful not to rupture the yolk. With the naked eye and under the dissecting microscope note the general plan of circulation (cf. chart) and the pulsation of the heart.
  - 2. Demonstration of the movement of the blood1 in the web

¹ In case a frog is used for this demonstration it should first be pithed and the part to be examined then pinned out, but not too tightly stretched, over an aperture in a sheet of cork and placed upon the microscope stage. Keep the parts moist with physiological salt. If a salamander be used, it may be anesthetized by placing it in a solution of chloretone and then examined under water in a watch glass or stentor dish under a binocular dissecting microscope or a low power of the compound microscope.

of the frog's foot, in the tongue of the frog, in the external gills of a Salamander larva, or in the unpigmented region of the skin of an adult salamander: Under the compound microscope the movements of the individual corpuscles may be seen. Note the relative rate in the capillaries, in the artery which brings the blood to the capillaries, and in the vein which carries it away.

- 3. Demonstration by means of the Circulation Scheme devised by Porter,<sup>1</sup> (or by some similar apparatus) of arterial and venous pressure and of the conditions affecting them. *Record the facts learned from this demonstration*.
- 4. Demonstration of the pulse tracing. By means of some form of sphygmograph (e.g., Dudgeon, Marey, or Jacquet),<sup>2</sup> make a pulse tracing from your own or another person's radial artery, and, after suitably labeling it, spray it with a weak shellac, or dip it in a shellac bath, to prevent rubbing, and mount it in the laboratory book, accompanied by an explanation of your interpretation. Note that the pulse tracing shows always a rapid ascent and partial descent, constituting the primary wave, followed by at least one smaller or secondary wave; the primary wave expresses the sudden expansion of the artery caused by the ventricular systole, and the secondary waves, the chief of which is known as the dicrotic wave, are due to the elastic vibration of the arterial walls.
- 5. Demonstration of arterial pressure and the method of measuring it by means of some form of sphygmomanometer. This instrument is usually applied to the upper arm of the subject and consists of a device for exerting upon the arm pressure which is transmitted through the tissues to the brachial artery, thus tending to obliterate the radial pulse which the operator is simultaneously keeping track of. The amount of pressure which is being applied is automatically registered and is cumulative, so that at the moment when the complete obliteration of the pulse

<sup>&</sup>lt;sup>1</sup> Porter, Introduction to Physiology, pp. 511-519, also Science, 1905, XXI, pp. 752-754. The demonstration apparatus here referred to is obtainable from the Harvard Apparatus Company. Since this apparatus or any similar one which might be used, would be accompanied by a description of the method of using it, it has been deemed sufficient for the purposes of these outlines, to merely give references to the apparatus and to any special descriptions of its use.

<sup>&</sup>lt;sup>2</sup> Beddard (and others), Practical Physiology, pp. 80-84.

occurs, the pressure which is registered equals the maximum or systolic arterial pressure. Make and record as many determinations of systolic arterial pressure as the time allows. To insure accuracy several determinations should be made upon the same subject by each student before any comparison can be made of the determination of arterial pressure of different individuals.

### XV. BLOOD

#### A. HISTOLOGY OF BLOOD.

#### 1. Examination of Fresh Blood.

Amphibian Blood.—This may be obtained from a freshly killed specimen of Necturus or from the cut surface left by snipping off the toe of a living frog.

Add a little of the blood to a drop of physiological salt solution upon the middle of a clean slide, and cover at once with a clean cover-slip. Examine first under low power, then under high power, of the compound microscope. Note the erythrocytes, their color, shape as seen lying flat and also upon edge, shape and position of nucleus. Draw flat and edge views. Note the far less numerous leucocytes, with their varying sizes, granular cytoplasm, pseudopodia, and nuclei. Watch a single leucocyte for 5 minutes making sketches of it at half-minute intervals to detect amæboid activity.

Human Blood.—This may be conveniently obtained and mounted as follows: Prepare two clean cover-slips. Sterilize the surface from which blood is to be drawn (the skin of the finger at the base of the nail, the ball of the finger, or the lobe of the ear) by washing thoroughly with 70% alcohol applied by means of a little absorbent cotton. After the surface has dried do not allow anything to come in contact with it until the incision has been made. Sterilize a clean, sharp needle or lance by rubbing it thoroughly with a little cotton wet with 70% alcohol. Allow it to dry. Make a quick cut deep enough to allow the blood to flow out freely, press slightly to start the blood, but do not continue to use pressure. Place a drop of the blood thus obtained upon one of the cover-slips. Immediately apply the other cover-slip to this, thus spreading the blood in a thin film. Lay this double cover-slip with the film of blood between, upon a glass slide and examine it first under low, then under high power. Note the erythrocytes, rapidly losing their smooth outline which becomes indented at regular intervals (crenated). On this account fresh

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preparations must be frequently made. Compare with the amphibian erythrocytes as to color, size, shape, and absence of nucleus. (This comparison may be made more conveniently and accurately if a mixed mount of human and amphibian blood in a drop of physiological salt solution be used.) By careful focusing, the thinner central region and thicker margin may be demonstrated. Note that the edge view exhibits a characteristic dumbbell shape, although under perfectly normal conditions the erythrocytes have been found to be concavo-convex, resembling half of a hollow sphere. Note the collection of the erythrocytes into rouleaux. Draw a few erythrocytes from different aspects.

Among the erythrocytes look for various kinds of leucocytes which, however, will be better distinguished from the stained preparations later. Note range of size, granules, nuclei. *Draw a few leucocytes*.

# 2. Smear Preparations of Human Blood, for the Study of Leucocytes.

Method of Preparation of Blood Smear.—The slide or coverslip upon which the film of blood is to be spread should be absolutely clean and free from grease. "Bon Ami" is recommended as an effective cleaning agent.

Take off directly upon the slide at about three quarters of an inch from one end, a drop of freshly drawn blood. Place the slide on the table and use a second clean slide as a spreader, by bringing one end of it in contact with the full width of the first slide on the side of the drop toward the middle of the slide and then slanting the spreader slide back toward the drop until, when it makes an angle of about 30° with the lower slide, it comes in contact with the drop which is thus made to flow across the slide in the angle between it and the spreader. The spreader, still held at an angle of 30° is then pushed steadily toward the opposite end of the slide, dragging the blood after it in the form of an evenly distributed film upon the middle region of the slide.

After spreading the film it should be carefully dried in air, care being taken not to allow anything to come in contact with it. Wright's stain, which is a mixture of eosinate of methylene blue and eosinate of methylene azur in a nearly saturate solution

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in pure methyl alcohol, is now poured carefully upon the horizontal slide until the film is just covered by it. After standing thus for one minute the stain is diluted by adding drop by drop from a pipette enough distilled water to equal two or three times the volume of the stain. The diluted stain should then remain from five to ten minutes and should then be washed off gently with water.

The slide should next be carried over in a horizontal position into a wide dish of distilled water for the purpose of differentiating the stain, and the washing continued until the more evenly spread portions of the film are yellowish or reddish in color. This should take from one to three minutes. The excess of water should then be drained off and absorbed by means of filter paper, and the preparation may then be put aside to dry. Since it is advisable to dry it as rapidly as posisble, the application of a very gentle warmth is recommended. When the drying is completed the slide is ready for examination.

The differential staining makes it possible to distinguish between the varieties of leucocytes, which will be found scattered among the masses of pinkish orange erythrocytes.

The following types should be identified (Cf. plates in reference books showing the differences between the various types):

- a. Small mononuclear leucocyte (lymphocyte), with clearly defined, dark purplish-blue nucleus and a small amount of bluishgreen cytoplasm in which a few reddish granules may be seen. These leucocytes vary in size from one to two times that of an erythrocyte, and constitute about 20%-30% of all the leucocytes of normal blood.
- b. Large mononuclear leucocyte with the same general staining reaction as the small ones but less intensely so. The nuclei are proportionately smaller and are more variable in form from rounded to irregular. In size this type is from two to three times that of the erythrocytes. They constitute a very small percentage, 1%-8%, of the leucocytes of normal blood.
- c. Three varieties of polymorphonuclear leucocytes, all with very variable, irregularly lobed or subdivided nuclei, which may even appear to be fragmented into separate masses. These cells vary in size from two to three times that of an erythrocyte. The three varieties, distinguished from each other mainly by the differential

staining of granules in the cytoplasm which otherwise does not take up the stain, are as follows:

Neutrophiles.—In these the nucleus takes a purple stain. The granules in the cytoplasm are so fine as to be hardly distinguishable as separate granules, so that the reddish-purple stain which they take (showing them to be neutral rather than either acid or basic in their affinities) seems to impart its color to the whole cytoplasm. These are the most abundant of all the types of leucocytes, constituting normally 60% to 70% of all the leucocytes.

Acidophiles or Eosinophiles.—These are characterized by the affinity for acids of the coarse granules in the cytoplasm. These granules in consequence take on a red color from the eosin and are thus conspicuous in an otherwise unstained cytoplasm which surrounds the purple nucleus. The acidophiles constitute only from 1% to 4% of the total number of leucocytes in normal blood, and are thus very difficult to find.

Basophiles.—In these the nucleus stains only faintly and is thus inconspicuous. The coarse granules in the cytoplasm have a strong basic affinity and thus stain deeply with methylene blue while they may also take on a purplish tinge similar to that imparted to the fine granules in the cytoplasm. The blue-stained, coarse granules are, however, so conspicuous that they seem even to stand out from the surface of the cytoplasm. This is the least abundant variety of leucocytes, since they constitute less than 1% of the whole number. They are therefore seldom seen in a single preparation.

Identify as many varieties as you are able to find and draw each variety identified.

3. The Blood Count by Means of the Hæmatocytometer.—Using the mount of human blood set up for this purpose, make the count of erythrocytes as directed by the method accompanying the apparatus, and compute from this the number of erythrocytes in 1 cu. mm. of blood. Record the entire computation.

### B. GENERAL PROPERTIES OF BLOOD.

### 1. Coagulation.

Take a quantity (50 c.c. or more) of freshly drawn blood, which, if obtained from the abattoir, must be kept at a low tem-

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perature during the transportation. Divide this into two parts. Set one portion aside, at ordinary room temperature, and, without disturbing it, observe from time to time during a period of half an hour or longer, the process of formation of the dark red clot. and its gradual shrinkage away from the sides of the container, leaving the yellowish serum as the liquid portion of the blood. Record your observations together with the exact time when each is made. Meanwhile, whip the second portion (25 c.c. or more) in a shallow evaporating dish, with a few twigs or broom corns, and note that as a result the formation of the threads of fibrin. which is an essential element in the process of clotting, is hastened, and that the agitation detaches the erythrocytes, which are normally held in the meshes of the fibrin, so that these remain in the serum when eventually the fibrin threads, clinging to the broom corns, are removed from the blood. Blood thus treated is known as defibrinated blood and remains indefinitely without clotting.

# 2. Determination of the Proportions of Plasma and Cells in Freshly Drawn Blood.

Use blood which has not begun to clot, or, if this is not available, defibrinated blood (the latter will give the proportion of serum, rather than of plasma, to blood cells). Carefully measure a sample of the blood to be used, in the graduated tube of a centrifuge machine, and after subjecting it to the centrifuging process sufficiently to drive the blood cells, which are heavier than the plasma, to the bottom of the tube, determine the proportion of these by volume (approximately) by reading the graduations corresponding to the various levels. *Record*.

# 3. Specific Gravity of Blood.

Determine the specific gravity of a drop of human blood by the following method: Place in a beaker a stock mixture of chloroform and benzol having a specific gravity of about 1055. Draw a drop of blood by the method already learned and shake it from the finger into the mixture. If it gradually sinks add chloroform drop by drop, stirring the mixture gently, until the drop of blood neither rises nor sinks but remains stationary at any level, thus

showing that its specific gravity is equal to that of the mixture. If, on the other hand, the drop of blood floats add benzol gradually and stir the mixture in the same way until a mixture is obtained in which the blood will remain suspended at any level. Then pour the mixture into a tall cylinder and determine its specific gravity with the hydrometer. This will also be the specific gravity of the blood. In returning the mixture to the stock bottle, remove the blood by straining. Record your method of procedure and the result of the determination.

4. Determination of the Percentage of Hæmoglobin in a Sample of Blood by Means of Some Form of Hæmoglobinometer. Follow carefully the directions for using this device. Record the percentage determined.

<sup>&</sup>lt;sup>1</sup> The Tallquist hemoglobin scale is a convenient form for this work.

### XVI. THE RESPIRATORY PROCESS

# A. LOCATION OF THE EXTENT OF THE LUNGS BY PERCUSSION.

For this exercise the subject should be loosely and thinly dressed. Place the index finger of the left hand upon various regions of the thoracic and abdominal wall of the living human subject, and listen to the sounds produced when the finger is tapped with the middle and ring fingers of the right hand. Note that over the lungs there is a hollow reverberating sound which is absent from other regions. Record the extent of the lungs upon outline drawings of the body, or upon the Suzuki manikin.

# B. CHANGES IN THE DIMENSIONS OF THE THORAX AND ABDOMEN OF THE HUMAN SUBJECT DURING RESPIRATION.

A group of students may conveniently work together in obtaining these statistics. The subject must be loosely and thinly dressed. The measurements of width (from side to side), and depth (from front to back), are taken with calipers (pelvimeter), the girth measurements with a tape measure, and the height above the floor with an anthropometer. The records should be made in millimeters, and should be based upon averages obtained from at least five independent measurements of the same subject.

#### 1. Horizontal Measurements.

# (a) At the Level of the Lower End of the Sternum.

	Girth	Depth	Width	Index d/w
At the end of a natural expiration				

(b) At the Level of the Umbilicus (i.e., just above the crest of the os ilium).

	Girth	Depth	Width	Index d/w
At the end of a natural expiration.  At the end of a natural inspiration.  At the end of a forced expiration.  At the end of a forced inspiration.				

### 2. Vertical Measurements.

(a) Lower End of Sternum.

(a) Donot Ditte of Storiation	
	Height above floor
At the end of a natural expiration.  At the end of a natural inspiration  At the end of a forced expiration.  At the end of a forced inspiration.	
(b) Distal End of Clavicle.	
	Height above floor
At the end of a natural expiration	

Make a summary of conclusions under four heads:

At the end of a natural inspiration

At the end of a forced expiration

At the end of the forced inspiration

- 1. The changes in the dimensions of the thorax during normal breathing.
- 2. The changes in the dimensions of the abdomen during normal breathing.
- 3. The changes in the dimensions of the thorax during forced breathing.
- 4. The changes in the dimensions of the abdomen during forced breathing.

Compare results obtained from as large a number of subjects as possible to show different types of respiration.

### C. PNEUMOGRAPH RECORD OF RESPIRATORY MOVE-MENTS.

A group of four students may conveniently work together in making these records. The subject should be dressed as for (A). Adjust the pneumograph to the chest at the level of the lower end of the sternum. Attach the tube to the tambour, the writing point of which rests very obliquely upon the smoked paper carried by a slowly revolving drum. When all is adjusted, one student may start the drum to revolve, and, with a stop watch, time the duration of the experiment to exactly one minute, at the end of which time the drum must be stopped. During this time the subject should face away from the apparatus, and should breathe normally, which may be best accomplished if he fixes his attention upon some subject foreign to the experiment. Separate records at different levels on the drum may be made for each student of the group. The paper may then be removed, the record carefully labeled and passed through a shellac bath. Dry by laying it, smoked side up, upon a sheet of filter paper. The records may then be cut apart, mounted in the laboratory book, measured and interpreted as to the following particulars:

- 1. Rate of respiration per minute.
- 2. Comparison of duration of the act of inspiration, expiration, and the post-inspiratory and post-expiratory pauses.
- 3. Comparison of steadiness of movement of inspiration and expiration.

# D. THE MECHANICS OF RESPIRATION (THE "RESPIRATION SCHEME" OF PORTER).<sup>1</sup>

Not more than two students can work conveniently with this apparatus at one time. Make the experiments as directed (Porter, pp. 506-507). Record the experiments in the form of definite complete statements as to the conditions and results.

<sup>&</sup>lt;sup>1</sup> This apparatus may be obtained from the Harvard Apparatus Company. See Porter's Introduction to Physiology.

### E. RESPIRATORY SOUNDS.

By means of the phonendoscope adjusted with the end of the rod applied (1) to the region of the trachea, (2) over the apex of one of the lungs (beneath the clavicle), and (3) to the back at one side of the midline at about the level of the fourth intercostal space, listen to the sounds accompanying respiration. Try to distinguish (1) sounds of the air moving through larynx and trachea and (2) vesicular murmurs which are due to the opening up of the bronchioles and alveoli during inspiration, and which die away during the latter part of expiration. The subject should be loosely and thinly dressed.

# F. SPIROMETER MEASUREMENTS OF THE VOLUMES OF AIR CONCERNED IN RESPIRATION.

At least five trials should be made for each measurement and the results recorded and averaged. Each student should make the records from his own respiration; two students may, however, conveniently assist each other in obtaining the records. Record the measurements in cubic centimeters (1 liter = 1000 cu. cm.).

- 1. The tidal volume, the amount breathed in and out in a normal respiratory act. Determine by measuring the amount of air breathed out in a normal expiration following a normal inspiration.
- 2. The complemental volume, the amount which is taken in during a forced inspiration in excess of the tidal volume. Determine by measuring the amount breathed out in a normal expiration after a forced inspiration and deducting the tidal volume already obtained.
- 3. The supplemental volume, the amount which may be expelled by a forced expiration in excess of the tidal volume. Determine by measuring the amount of air breathed out in a forced expiration following a normal inspiration and deducting the tidal volume already obtained.
- 4. The vital capacity (complemental plus tidal plus supplemental volumes). Determine by measuring the amount of air breathed out by a forced expiration following a forced inspiration. Check by comparison with the sum of 1, 2, and 3.

- G. THE DEMONSTRATION OF THE NATURE OF EXPIRED AIR.
- 1. The Excess of Carbon Dioxide.—This may be demonstrated by two bottles connected with a mouthpiece which is so arranged that the inhaled air passes through lime water in one bottle and the exhaled air through lime water in the other. This arrangement is made by using as a mouthpiece a Y-tube, one arm of which is connected by means of rubber tubing with a glass tube which passes through the tightly fitting cork of one bottle, reaching nearly to the bottom of the bottle, while the other arm of the tube is connected with a glass tube which leads only a short distance below the cork fitted tightly into the other bottle. The first bottle has an open glass tube leading through its cork from the upper part of the bottle, while the second bottle has another glass tube leading from near the bottom of the bottle. Each bottle is filled half full with filtered lime water at the beginning of the experiment. A rubber tube attached to the stem of the Ytube serves as a mouthpiece, and can be washed or renewed as needed. Breathe in and out through this apparatus for a short time and note the result. Milkin is induced in lime water by a stream of gas passing through it is a test for carbon dioxide in the gas. Record the result, accompanying it by a diagram of the apparatus. Finally empty the lime water out and thoroughly rinse each bottle, using a weak solution of hydrochloric acid if necessary.
- 2. The Deficiency of Oxygen.—Invert over water, with its mouth submerged, a wide-mouthed bottle filled with air. By means of a bent tube passed under water and into the bottle breathe the contained air in and then out two or three times. Lift the bottle and, keeping its mouth down, quickly put a lighted taper into it. As a control burn a taper in a similar inverted bottle filled with air. A flame will not burn in an atmosphere of less than 17% of oxygen, i.e., a tension equal to 129 mm. of mercury. Record your conclusions.

### XVII. THE DIGESTIVE SYSTEM

#### A. GROSS ANATOMY.

By means of dissected demonstration preparations¹ (cats, rabbits, or other mammals) supplied for this study, review the gross anatomy of the digestive system (cf. pp. 11-13) and record by drawing such details as have not been previously recorded in your work.

Mouth.—Note its extent and boundaries; character of the cheeks, lips, teeth, roof of mouth, and tongue; the salivary glands (parotid, submaxillary, and sublingual) and the ducts leading from these to the mouth.

**Pharynx.**—Note extent; walls, especially the lateral walls in which the tonsils are located; various orifices opening into and from it as identified in previous study of the sheep or calf (cf. p. 86).

**Œsophagus.**—Note relation to larynx and trachea; course through thorax and relation to other organs of the thorax; the muscular nature of its walls; its collapsed condition when empty; the character of its lining; the passage of its posterior end through the diaphragm to reach the stomach.

Stomach.—Note its location with relation to the diaphragm and to the other abdominal organs; the extension of its mesentery to form the overhanging greater omentum and the relation of this to the spleen; the differentiation of the stomach into cardiac and pyloric regions; the pouch-like form of the cardiac region, with its fundus bounded by the greater and lesser curvatures; the

<sup>1</sup> These preparations may be made very conveniently by detaching from the cœlomic wall the entire length of alimentary canal, together with its associated glands, the heart, the lungs, and as much as possible of the diaphragm, all held in their normal relationships by the serous membranes. In the neck region the œsophagus and trachea should be detached from the adjacent muscular and skeletal parts and the neck severed near the base of the skull. The head is thus kept in its relationship to the alimentary canal, while the rest of the body may be discarded. Further dissection may then be made to show salivary glands and ducts and the ducts of the liver and pancreas. In each region of the alimentary canal a sufficient length should be laid open to make possible the examination of the walls and lining.

tubular form of the pyloric region; the pyloric sphincter muscle guarding the exit into the intestine; the muscular walls, and the deviation from the strict longitudinal and circular arrangement of muscle fibers thus giving rise to oblique layers; the character of its mucous lining with the numerous folds, an accommodation to the distensibility of the organ.

Liver.—Note its location, voluminous size, and the form and arrangement of its lobes; the folds of the peritoneum supporting the liver and attaching it to the diaphragm (falciform ligament); the lesser omentum stretching between the liver, and the pyloric end of the stomach and anterior end of the intestine; the position of the gall bladder with ducts leading into it from the liver and the cystic duct leading from it into the anterior region of the intestine (duodenum).

Pancreas.—Note its thin and irregular form due to its location within the mesentery between the stomach and duodenum, into which its duct leads.

Small Intestine.—Note its length, involving convolutions and an enormous expansion of the ventral edge of the mesentery which supports it; the numerous blood vessels, lymph glands and vessels, fat deposits, and (especially conspicuous in the cat) small glistening Pacinian corpuscles between the two layers of the mesentery; the muscular coats strongly differentiated into the longitudinal and circular layers; the velvety appearance of the mucous lining due to the villi which thickly stud its surface; the circular folds (valvulæ conniventes) of the mucous lining; the differentiation of the first loop of the intestine as the duodenum, and the relation of this to the pancreas and its duct as well as to the cystic duct; the opening of the small intestine into the side of the large intestine (location of ileo-colic valve).

Large Intestine.—Note its wider caliber; the blind end (cæcum) extending beyond the opening of the small intestine into it (cf. the voluminous cæcum of rodents, and the reduced cæcum and the vermiform appendix of man); its differentiation, less clearly marked than in man, into ascending (right side), transverse, and descending colons, and rectum; thinner nature of the muscular walls, as compared with those of the small intestine, the absence of villi, and the smoother nature of the lining.

#### B. PHYSIOLOGICAL DEMONSTRATIONS.

- I. Peristalsis.—Use a recently chloroformed or etherized frog, Necturus, or mammal (preferably the latter) from a half hour to an hour after feeding. Expose the intestines by opening the abdominal cavity. Observe the waves of contraction which pass slowly along the intestine and note the changes of form which they involve, and their effect in producing an onward movement of the contents of the region which is under observation. Note that these movements may be induced by mechanical stimulation.
- 2. Appearance of the Food in Different Regions of the Digestive Tract during the Process of Digestion.—The specimens (cats and rabbits) used for this demonstration should be killed about a half hour or an hour after feeding. Open various regions of the digestive tract and compare the condition of the food material found in different regions, also note the conspicuous differences which result from the difference in normal diet in such animals as the cat (carnivorous) and the rabbit (herbivorous).

Note particularly the slightly digested and thus still recognizable food substances in the stomach, the creamy chyle in the small intestine, and the gradual accumulation of fæcal matter which is left after the absorption of the nutritive portions of the food and which becomes more abundant and offensive in odor as a result of bacterial decomposition as the posterior region of the intestine is reached. Note also the degree to which the fæces are moulded into characteristic shapes before expulsion. Note, incidentally, the distended lymphatic vessels (lacteals) of the mesentery and follow these to the receptaculum chyli and thoracic duct (especially well seen in a cat which has been fed with rich milk about half an hour previous to chloroforming).

#### C. HISTOLOGY.

# 1. The Alimentary Canal.

**Macroscopic Study.**—In preparation for the microscopic study of the intestine, examine macroscopically a short length of intestine (cat) which has been thoroughly hardened in formalin or some other fixing agent. With a sharp-scalpel make a clean transverse section of this and examine the cut end. Tease or tear apart the various layers. Note (a) the visceral peritoneal layer which

covers the whole outer surface forming the very thin serous coat (tunica serosa); within this, (b) the thick, firm muscular coat (tunica muscularis) consisting of a thin outer layer of longitudinal fibers which may be easily stripped off (together with the serosa), and a thicker inner layer of circular fibers; inside the muscularis (c) a layer of connective tissue (tela submucosa); and finally (d) the innermost layer (tunica mucosa) which is very conspicuous because of the innumerable absorbent organs (villi) which thickly cover its surface and project into the lumen. Note the velvety appearance which the inner surface presents when a short length of intestine is laid open and washed.

Microscopic Study (Lab. Sl. Coll.) - Examine cross sections of the intestine of an amphibian and identify the various coats above mentioned. Note that the serosa is continuous with the mesentery and consists of a single layer of flat cells showing in cross section as a thin line with occasional flattened nuclei; that the spindle shaped involuntary muscle fibers (each a single cell with a single nucleus) of the longitudinal layer are cut transversely and those of the circular layer are cut longitudinally; that there are numerous blood vessels in the various layers, particularly in the submucosa; that the mucosa has no villi as has the mammalian intestine but is thrown into numerous longitudinal folds, here cut transversely, which would disappear if the intestine were distended with food; that these folds are covered by a simple layer of columnar epithelial cells among which are many cells of the goblet type, i.e., cells which pour out their mucous secretion from time to time into the intestine; that there are a few multicellular intestinal glands of the coiled tubular type, lined with simple epithelium and opening into the intestinal lumen between the folds. Draw the whole section on as large a scale as the size of the page will allow, and show the outlines of the various layers, filling in, in their proper places, representative details of cell structure.

Examine cross sections of the small intestine of the rabbit, for general identification of layers. Note that here the muscular layers are very thin; that the mucosa possesses in addition to a few large folds, numerous villi, and a much larger number of intestinal glands, which are also larger and more complicated.

Examine longitudinal sections of the small intestine of the dog or cat. Note that the sections are so curved by the contraction of the muscularis that the mucosa lies on the outer border of the section, the villi well separated. Under low power identify the layers, noting the occurrence of an additional thin muscle layer between the mucosa and the submucosa known as the muscularis mucosæ; under high power study the details of villi and glands. Draw details showing (1) a longitudinal section through a villus, and (2) the form of a group of intestinal glands and their openings into the lumen of the intestine. Occasionally compact masses of lymphoid tissue, known as solitary lymph nodules, will be seen in the submucosa.

Study a preparation showing cross sections of individual villi. Draw one or two, as seen under high power.

Study again cross sections of **injected intestine** to show the network of capillaries in the various layers, particularly in the villi.

Study sections through various regions of the wall of the stomach. Note the same general arrangement of coats as in the intestine, with modifications in the form of oblique layers in the muscularis; the numerous deep folds of the mucosa, with tubular glands opening between them, peptic glands, of the simple or slightly branching tubular type in the region of the fundus, and pyloric glands, more branching and more convoluted, in form, in the pyloric region. Draw a view showing the general arrangement of layers as seen under low power; draw details of folds and glands of mucosa of the fundus and of the pyloric region under high power.

Study sections of **injected stomach** to show capillary network, particularly of the mucosa.

### 2. Special Digestive Glands.

The primitive glandular structure, already studied in the case of the glands of the stomach and intestine, consists of an invagination of the epithelium, in the form either of a tube or of an alveolus, lined throughout with epithelial cells. When the invagination becomes so extensive and so complicated by branching that it forms a mass of tissue lying quite outside of the wall of the organ from which it arose, a distinct organ such as a salivary gland, pancreas, or liver is formed. Such an organ may retain its connection with the epithelium from which it was derived, through a more or less elongated duct, the lumen of which on the one hand leads from the lumina of the various tubules or alveoli of the gland, and on the other hand opens into the lumen of the alimentary canal. The extensive and repeated branching of a gland gives rise to lobes which are thus composed either of masses of tubules convoluted for greater compactness, or of branched tubules ending in alveolar expansions (acini), or of clusters of alveoli, according to the nature of the gland. Each ultimate alveolus or tubule will thus communicate with the intralobular duct, and these in turn will lead into interlobular ducts which lead finally into the common duct of the gland.

Salivary Gland (compound tubulo-alveolar).\(^1\)—Review the general external form and appearance of a salivary gland with its many lobes and its discharging duct, noting its investment of connective tissue which holds the lobes together and conveys the blood vessels and nerves to the gland tissue.

Study microscopically, sections of a salivary gland. Note the division into lobes, each exhibiting a compact mass of sections through the tubules and alveoli, among which may be seen occasional sections through the intra lobular ducts and their branches. Observe that the parts are held together by loose connective tissue, which is the interstitial tissue as distinguished from the parenchyma or true glandular tissue. Study under high power for details of structure as are peculiar to the gland in question, such as the form of lumen, the shape, size and arrangement of cells, nature of nucleus and cytoplasm, in the case both of the alveoli and of the ducts, and the distribution in the tubules and acini of serous and mucous cells. Note the presence of the zymogen granules in the serous cells, ready to be poured out as the liquid secretion of the gland. *Draw such details as you see clearly and understand*.

Liver (modified type of tubular gland).—Examine macroscopically pieces of pig liver hardened in alcohol or formalin, make fresh sections of it in various directions and note that it is a compact

<sup>&</sup>lt;sup>1</sup> Sections of the pancreas may be substituted for this study, as a type of tubuloalveolar gland.

mass made up of numerous lobules, each having somewhat the form of two frusta of pyramids placed base to base and thus appearing in section in irregular polygonal form. Study microscopically, sections of the liver of various mammals (the pig particularly), for the relation of the lobules to each other and the identification of blood vessels and bile ducts shown in a transverse section of a lobule as follows: Running through the center of each lobule is a small vein (the intralobular vein) which collects the blood from the capillaries of the lobule, while in the connective tissue at the angles between lobules (interlobular) are the ultimate branches of the hepatic portal vein and the hepatic artery, both of which bring the blood to the capillaries of adjoining lobules. With these interlobular blood vessels are found the bile ducts which convey the bile, the secretion of the liver cells, away from the bile capillaries (i.e., lumina of tubules) of the adjoining lobules. Note that the veins and arteries may be distinguished from each other by the character of their walls, the arteries having the thicker walls and the lining thrown into folds. Note also that the cuboidal epithelial cells lining the bile ducts distinguish them from both veins and arteries. Study sections of injected liver to corroborate these points. Draw a cross section of a single lobule showing its relation to adjoining lobules, and the arrangement of blood vessels and bile ducts associated with it.

Under high power study the details of liver cells. Note their cuboidal form, nucleus and cytoplasm, the latter often exhibiting numerous glycogen granules and other non-living structures. The bile capillaries which are really the lumina of the gland tubes which make up the liver, are distinguishable here and there merely as clefts beween the liver cells; the blood capillaries have their own lining of flat endothelial cells, and in injected liver, may be seen among the gland cells. (Compare with sections of Amphibian and reptilian liver in which the cells are larger and the structure simpler. Note particularly that in the reptilian liver the tubular character of the gland is very evident.) Draw a few adjoining cells showing all the details you can make out.

#### XVIII. THE UROGENITAL SYSTEM

#### A. EXTERNAL FORM AND RELATIONSHIPS.

From demonstration preparations, review the general identification of the urogenital organs, in situ, in both male and female of several species of mammals (cf. pp. 13-15). Draw such details of the relationships in each sex as have not previously been recorded.

- r. Urinary (Uropoietic) Organs.—Kidneys, their location (note which is more anterior, cf. man), relation to peritoneum, form, blood supply; suprarenal glands, their relation to the kidneys; ureters, relation to the kidneys and bladder; bladder, its location with reference to other pelvic organs, and to peritoneum (folds of which form the ventral suspensory ligament and two lateral ligaments), the location of orifices of ureters and urethra, the appearance of the walls of the bladder, both in the distended, and contracted conditions, the presence of muscular and mucous coats (the latter seen by opening the organ); urethra, its short course in the female to the external orifice in the urogenital sinus, its prolongation and specialization in the male into the organ of copulation (the penis), represented in the female by the clitoris.
- 2. Female Genital Organs.—Ovaries, their location, relation to peritoneum (note that each is enclosed, together with the uterine tube and uterus, in a fold of peritoneum known as the mesovarium or broad ligament), form, size; uterine tubes (oviducts), relation of the funnel-shaped mouth of uterine tube to ovary (demonstrate by probing with a wax-tipped bristle), and the coiled course of the tube to reach the uterus; uterus, its location with reference to other pelvic organs and to peritoneum, its division into two lateral cornua and a median body (in the rabbit, two separate uteri) leading posteriorly into the vagina, and its thick wall consisting of muscular and mucous coats; vagina, with thinner walls, leading posteriorly to the external orifice dorsal to the urethral orifice within the urogenital sinus. Note the midventral elevation of the wall of the urogenital sinus, known as the clitoris.

3. Male Genital Organs.—Note location of the testes in the scrotal sacs and the muscular and peritoneal elements which enter into the walls of the latter and enclose the testes themselves. The removal of the visceral peritoneal layer discloses the testis in its capsule, and its associated structure, the epididymis, the tubules of which form the connection between the testis and the ductus deferens through which its secretion is discharged. Note the course of the ductus deferens through the inguinal canal as one component of the spermatic cord. Demonstrate the relation of the ductus deferentia, seminal vesicles, and prostate and bulbourethral glands to each other and to the urethra. The penis is characterized by the development of three columns of erectile tissue (i.e., tissue containing large blood sinuses, which, when distended with blood, produce the erect condition necessary to copulation) as follows: The median corpus cavernosum urethræ in the posterior region, ending in the enlargement known as the glans, and the two lateral corpora cavernosa penis, all withdrawn distally within the prepuce, when the penis is not in a state of erection. Note also certain muscular elements which arise from the ischium and are inserted into the penis.

#### B. STRUCTURE.

## 1. The Kidney.

Macroscopic Study.—Examine externally a simple kidney such as that of a rabbit or guinea pig, carefully removing the loose packing of fat and areolar tissue in which it is imbedded. Note the tough capsule covering it, and the depression or hilus through which the ureter and the renal blood vessels pass. Cut the kidney by a longitudinal section passing through the hilus and identify the following regions and parts: The granular cortex; the medulla, with striated appearance converging from the cortex to the apex of a large papilla; the cavity or pelvis into which the papilla projects embraced by the expanded funnel-shaped calyx from which the ureter leads; and the veins and arteries outside of the calyx and ureter, within the sinus of the kidney. Since the ureter, calyx, and blood vessels are held together within the sinus by a packing of fatty areolar tissue, this must be removed in order to

show these structures. Draw a longitudinal section showing the plan of structure of a simple kidney.

For comparison with this simple kidney of the rabbit study the compound kidneys (1) of the cow or calf where the components are more or less separated, and (2) of the sheep and pig where the components are closely fused as in the human kidney. Draw a diagram of each type showing the relation of the components.

Microscopic Study (Lab. Sl. Coll.).—The real secreting organs of the kidney are the very numerous and complicated renal tubules. Each tubule begins with (a) the glomerulus capsule, which consists of a spherical expansion rendered two layered by the pushing in of one side through the ingrowth of a knot of blood vessels (a glomerulus). These are distributed through the cortex and form the renal corpuscles. From this capsule leads (b) the convoluted tubule, situated also in the cortex, and leading into (c) the straight tubule, which forms a loop, consisting of a thinner walled descending and a thicker walled ascending region, extending into the medulla and back into the cortex, where it leads into (d) the intercalated piece, a second convoluted region, which leads into (e) a collecting tubule, into which many other renal tubules open; this passes through the medulla to reach (f) a papillary duct which opens upon the free surface of the papilla by a pore through which the secretion (urine) is constantly drained off into the calyx, to be carried from the kidney through the ureter.

Study under low power a section of **rabbit kidney**. Identify the cortical and medullary regions, and note the general course of the tubules in each. *Draw the whole section to show its general topography as seen under low power*.

Study under high power each region to identify (1) the capsules of the glomeruli with the lining of flat epithelial cells and the beginning of the convoluted tubule, and (2) sections of various regions of the renal tubules. Draw details, showing characteristic form of the epithelial cells of as many regions as you are able to identify (cf. illustrations and description in any good text-book of histology).

Study for comparison, sections of the kidneys of other mammals, including man, also sections of injected kidney, noting that the knot of blood vessels of a glomerulus is not a true capillary since the efferent vessel continues as an arteriole to finally break up into the capillary network of the kidney.

## 2. The Ovary (Lab. Sl. Coll.).

Study either macroscopically or with the aid of a dissecting microscope, slides showing sections through the **ovary of the cat, rabbit,** or **other mammal.** Note the general shape and appearance of the section and the location of the hilus (the region of attachment to the body wall and of entrance and exit of blood vessels and nerves).

Examine under low power of the compound microscope and identify (a) the vascular supporting framework or stroma, of a richly cellular connective tissue, which radiates from the hilus toward the periphery between certain large oval structures, the vesicular ovarian follicles (Graafian follicles); (b) the peripheral region or cortex, in which are located the various early stages of the developing germ cells (ova), each enclosed within a follicle which consists of one or more layers of cells; (c) the germinal epithelium (often injured in making the preparations), which consists of a single layer of epithelial cells covering the surface of the ovary; and (d), in ovaries of sexually mature animals, larger or smaller homogeneous areas each of which is a section through a corpus luteum, the structure which for a time occupies the space left by the rupture of a mature follicle to discharge the ovum from the surface of the ovary. Draw a view of the whole section showing the general topography.

Under low and high power study various stages in the **development of the ovarian follicles** as follows:

- (a) A primary ovarian follicle (young material is necessary for this): This consists of a group of cells which has sunk into the stroma from the germinal epithelium; one cell of the group develops into the germ cell (the ovum), the others arrange themselves in a single layer about the ovum and form the nutritive layer known as the follicular epithelium. Draw a section through a typical primary follicle, showing the cell arrangement.
- (b) A vesicular ovarian follicle: This consists of (1) an outer covering developed from the stroma and known as the theca folliculi, (2) several layers of follicular epithelial cells lining the

follicle and forming the stratum granulosum, (3) a thickened mass of follicular epithelial cells surrounding the ovum and forming the cumulus oöphorus, and (4) a fluid, the liquor folliculi, which fills the cavity of the follicle and becomes finally very great in amount (to serve the mechanical function of carrying out the ovum when the follicle ruptures).

Select with care a good section through **an ovum** in a large follicle, and study the ovum to identify (a) the cell membrane or zona pellucida, (b) the cytoplasm containing yolk (small in amount in the mammals) and constituting the vitellus, (c) the nucleus, known as the germinal vesicle, and (d) the nucleolus, known as the germinal spot. Draw a typical section through a large follicle, showing its parts and the parts of the ovum.

#### 3. The Testis (Lab. Sl. Coll.).

Study under the low power of the compound microscope, transverse sections of the mammalian testis, stained by some method which will bring out cells in mitosis. Note that the section consists of a large number of smaller sections, mainly transverse, of the numerous seminiferous tubules of which the testis is chiefly made up while there is also a small amount of intertubular connective tissue in which are present large cells known as the interstitial cells. In the walls of the tubules all stages of developing male germ cells may be seen. Carefully select for study under high power sections of different tubules which show the following stages of development of the male germ cells, noting particularly which of these stages occur simultaneously in a given tubule:

- (a) Spermatogonia, the small cells which lie in contact with the basement membrane of connective tissue forming the outer wall of the tubule.
- (b) Primary spermatocytes, formed by the growth of certain of the spermatogonia.
- (c) Secondary spermatocytes, formed by the division of each of the primary spermatocytes into two daughter cells; they are therefore half the size of the primary spermatocytes and twice as numerous.

- (d) Spermatids, formed by the division of the secondary spermatocytes; they are half the size of the latter and twice as numerous, and lie almost or quite detached in the lumen of the tubule until they begin to elongate and reach in clusters between the columns of spermatogonia and spermatocytes in the wall of the tubule, and attach themselves to certain large pyramidal nutritive cells which lie on the basement membrane and are known as the Sertoli cells. These support and nourish the spermatids during their transformation into spermatozoa.
- (e) Spermatozoa, the fully developed male germ cells; these are greatly elongated, motile cells with lance-shaped heads (in the rat) in which the nucleus is located, and a long filamentous tail which serves as the organ of locomotion, by means of which the cells move freely in the tubules and the ducts. (Cf. living spermatozoa of rat, guinea pig, or some amphibian, and demonstration slides showing human or other spermatozoa.)

Draw details of various tubules to show as many as possible of the stages in the growth and development of the male germ cells.

#### XIX. THE DEVELOPMENT OF MAMMALS

# A. DEMONSTRATION OF THE GRAVID UTERUS IN RODENTS AND CARNIVORES.

- **1. General Examination** (guinea pig, rabbit, cat, preferably fresh specimens).—Note the greatly enlarged veins and arteries indicating a corresponding increased blood supply; the number of embryos present, their size, and their location in the uterus. Draw a view of the uterus showing the number and location of the embryos.
- 2. Extraembryonal Structures and Their Relation to the Embryo.—After carefully opening the uterus in the region of one of the embryos, identify (a) the chorion, a sac which encloses the embryo and is closely applied to the lining of the uterus, from which it can, however, be separated in most cases, in toto; (b) the placenta, either discoidal or zonary, a vascular structure attached to the inner wall of the uterus, formed in part from its lining mucous membrane and in part from a differentiated region of the chorion, and hence supplied with two sets of blood channels, one connected with the maternal and the other with the embryonal circulatory system, (c) the umbilical cord passing from the placenta to the embryo and containing the veins and arteries which connect the placenta with the circulatory system of the embryo. and (d) the amnion, a thin transparent membrane which forms a sac enclosing the embryo and containing the amniotic fluid. Draw one or more diagrams showing the relation of the embryo to the extraembryonal parts.

#### B. DEMONSTRATION OF LACTATION.

In the various specimens of the gravid female guinea pigs studied note the condition of the mammary glands, and their increasing size with the advance of pregnancy. Examine a fresh dissection of a specimen in lactation (after the birth of the young) and demonstrate by an incision into the gland tissue, the presence of an abundance of secretion (milk) in the gland. Note also the enlarged condition of the nipples in which the gland orifices are located.

#### C. DEVELOPMENTAL STAGES.

By means of the dissecting microscope, study a series of stages of mammalian embryos (mouse, rat, cat, or pig) and follow out the development of trunk, head, limbs, and viscera (exposed by previous dissection). Draw three stages each magnified five diameters.

# D. DEMONSTRATION PREPARATIONS OF HUMAN (OR OTHER PRIMATE) MATERIAL.

- 1. Reproductive system with gravid uterus laid open to show the discoidal placenta, the amnion (closely adherent to the chorion), and the long twisted umbilical cord extending from the placenta to the embryo. *Draw*.
- 2. Chorion of early human embryo before the development of the placenta, showing chorionic villi. *Draw*.
- 3. Various stages of human embryos showing changes of form and proportions during development. (Cf. other mammalian embryos, and various atlases of human and mammalian embryology.) Identify any extraembryonal parts which may be attached to these specimens. Draw any which illustrate points not already recorded.

## SUMMARY OF EQUIPMENT AND MATERIAL

#### I. MANIKINS AND MODELS.

At least one good life-size anatomical manikin, capable of as complete dissection as possible. (The best available are probably those manufactured by Montandon, the successor of Auzoux.)

The Suzuki black-board manikin (of Japanese manufacture, cf. footnote p. 1, for superficial anatomy).

Model of dissected torso (human), life size, preferably one of each sex, for use in the laboratory for the demonstration of the thoracic and abdominal viscera.

Small plaster muscle casts of the whole figure in various poses (e.g., the Houdon muscle figure). These are inexpensive and are very convenient for demonstration in the laboratory to small groups of students, and for practice in recognizing superficial bony landmarks and muscles.

Models of the human brain, natural size and capable of dissection. Enlarged models of the human brain showing relations of fiber tracts (that of Dr. Florence Sabin is to be particularly recommended).

Various models of lower vertebrate brains.

Models of the human head showing sagittal sections, and topographical relations of the pharynx.

Model, enlarged, of the human larynx and its various parts.

Model of the human eye and its surroundings, enlarged.

Model of the human ear, enlarged, showing details of labyrinth.

Models of male and female pelves, the latter with gravid uterus and several stages of embryos.

Miscellaneous models showing such structures as the detailed musculature of various parts, the articulations of bones, or the enlarged details of structure of various organs of the body.

# II. SKELETONS, AND SKELETAL PREPARATIONS.

At least two correctly mounted human skeletons, one male, the other female.

One complete disarticulated human skeleton for each group of from two to four students in a laboratory division.

Additional skulls sufficient to supply each student in a laboratory division with one. About half of these should be sawn horizontally and at least one or two sagittally. Each skull should always be accompanied by, and handled upon, a small cushion (about 10 inches square) half filled with bran, which not only saves much wear and tear of the skull but also supports it firmly in any desired position.

One or more completely disarticulated human skulls, either mounted or unmounted.

A series of human skulls of different ages from birth to senility. Preparations showing the natural ligaments of various articulations of the human skeleton.

A preparation of the human skull showing the course of the cranial nerves to the various parts of the head.

Mounted skeletons of other mammals, such as the rabbit. cat. sheep, and several species of monkeys, including at least one higher anthropoid.

Skeletonized anterior and posterior appendages of the rabbit, pig (and as many other ungulates as possible). These may be student preparations which each class may add to for the benefit of the next class (cf. p. 52).

Separate bones, and fragments of bones, of as large a variety of mammals as possible, including man.

# III. DEMONSTRATION DISSECTIONS OF A MORE OR LESS PERMANENT NATURE.

(Most of these may be made in the laboratory and may be kept and used from year to year with occasional renewal and additions.)

Transverse sections through the middle of the trunk region of the dog fish.

Median sagittal and transverse sections through whole bodies of small mammals or advanced embryos.

Dissections of young cats or kittens to show the brain and spinal cord with the roots and divisions of the spinal nerves in situ.

Dissections of cats or other mammals to show the brachial and lumbosacral plexuses *in situ*.

Dissections of cats or other mammals to show the chain sympathetic ganglia and their connections with the visceral divisions of the spinal nerves.

Dissections showing the brain of the dogfish, and of other vertebrates, particularly the more primitive classes in situ.

Dissections of cats or other mammals with circulatory system, injected, for comparison with those forms used in the laboratory, or as a substitute (if time is limited) for specimens injected by individual students.

Dissections of cats, rabbits, or other mammals to show the relationships of the various parts of the digestive system.

Dissections of cats and rabbits to show the urogenital systems of males and females of each species.

Preparations of gravid uteri, preferably of some Primate.

Sets of mammalian embryos of various stages (pig embryos are readily obtainable), for the study of the development of external form.

A series of human embryos of different stages, some, at least, with the placenta and other extraembryonal parts attached.

Miscellaneous preparations, especially of parts of the human anatomy, such as dissections of the tongue and larynx, heart, etc.

#### IV. SLIDE COLLECTIONS.

Sets of slides (laboratory slide collection) in sufficient numbers to supply individual students of a laboratory division. The following are suggested as desirable:

- A. Mitosis in tissue cells.
  - T. Growing tips of onion root, longitudinal sections, stained with iron hæmatoxylin.
  - 2. Epidermis of salamander, surface mount, conjunctiva of eye, stained in hæmatoxylin or safranin.
- B. Epithelial tissues.
  - 1. Simple epithelium.
    - (a) Cuboidal: sections of gall bladder of Necturus.

- (b) Columnar: (1) Transverse sections of the stomach and gastric diverticula of a grasshopper; (2) Transverse sections through some simple type of vertebrate intestine; (3) Sections through the mammalian oviduct.
- (c) Squamous: Surface mounts of the mesentery. (Cf. also serosa in the transverse sections of vertebrate intestine.)

## 2. Stratified epithelium:

- (a) Cuboidal: Sections of larval amphibian skin.
- (b) Columnar: Sections of œsophagus of lungless salamander (Desmognathus, Eurycea).
- (c) Squamous: (1) Sections of adult amphibian skin, unpigmented region (Desmognathus, Eurycea) showing also simple alveolar glands; (2) Surface mounts of moult layer of adult amphibian skin; (3) Sections of human fetal skin; (4) Sections of lining of mouth of some mammal; (5) Sections through mammalian œsophagus.

#### C. Skeletal tissues.

- I. Tensile varieties.
  - (a) Loose: (1) Use sections of various organs such as intestine; (2) Adipose tissue of mesentery, surface mounts of thin regions.
  - (b) Dense: (1) Longitudinal and transverse sections of tendons; (2) Teased preparations, longitudinal, and transverse sections of elastic ligament (ligamentum nuchæ).
- 2. Rigid varieties.
  - (a) Cartilage: (1) Sections of hyalin cartilage; (2) Sections of elastic cartilage; (3) Sections of fibro cartilage.
  - (b) Bone: (1) Transverse sections through the decalcified shaft of a long bone; (2) Transverse sections of dry human bone (shaft of long bone); (3) Longitudinal sections of dry human bone (shaft of long bone); (4) Longitudinal sections of growing bone (end of a long bone).

#### D. Muscle tissues.

- I. Smooth involuntary muscle: Teased preparations (Use also sections of intestine for longitudinal and transverse sections of smooth muscle *in situ*.)
- 2. Striated voluntary muscle.
  - (a) Teased preparations.
  - (b) Longitudinal and transverse sections of voluntary muscle of Necturus.
  - (c) Longitudinal and transverse sections of voluntary muscle of rabbit or other mammal.
- 3. Striated involuntary, or heart, muscle: Longitudinal sections through a piece of heart muscle.

#### E. Nerve tissues.

#### I. Cells.

- (a) Smear preparations of nerve cells of spinal cord.
- (b) Golgi preparations of cerebral and cerebellar cortex.
- (c) Sections through cerebral and cerebellar cortex to show details of structure of the various types of cells in situ.

#### 2. Fibers.

- (a) Teased preparations of nerve fibers stained in hæmatoxylin.
- (b) Teased preparations of nerve fibers treated with osmic acid.
  - (c) Transverse sections of nerve fibers stained with hæmatoxylin.
  - (d) Transverse sections of nerve fibers treated with osmic acid.

# 3. Nerve endings.

- (a) Motor end plates in voluntary muscle.
- (b) Sensory endings (spindles) in voluntary muscle.
- (c) Pacinian corpuscles (in pancreas of cat).
- (d) Taste buds in the foliate papillæ of the rabbit.
- (e) Preparations showing the olfactory and epithelial cells of the nasal mucous membrane.
- (f) Sections of the retina (frog, rabbit, or other form).
- (g) Sections through the spiral organ of Corti (ear of pig, or other mammal).

#### F. Organs.

- 1. Transverse sections of spinal cord.
- 2. Sections of injected tissues and organs for the study of capillaries (e.g., intestine, tongue, lung).
- 3. Transverse sections through arteries and veins.
- 4. Transverse sections of the intestine of Necturus.
- 5. Transverse section of mammalian intestine (rodent recommended).
- 6. Longitudinal sections through mammalian intestine (carnivore recommended).
- 7. Sections through the various regions of the wall of the stomach.
- 8. Sections through the salivary gland (or the pancreas).
- 9. Sections of liver (pig and cat).
- 10. Sections of Necturus liver.
- 11. Sections of simple (rabbit) kidney.
- 12. Sections of compound kidney (man).
- 13. Sections of young and of mature mammalian ovaries.
- 14. Sections of mammalian testis.

#### G. Blood.

Smear preparations of human blood stained with Wright's stain.

Specially selected slides (demonstration slide collection), to be accompanied by suitable references or descriptions, and usually arranged under the microscope by the teacher or demonstrator.

- A. Examples of types of cells from various tissues, showing cell differentiation.
- B. Examples showing details of mitosis. (Ascaris eggs are especially good.)
- C. Special preparations of different tissues and organs for finer details of structure.
- D. Sections through various organs which are not otherwise made objects for histological study.

#### V. APPARATUS AND INSTRUMENTS.

A dissecting and a compound microscope for each student in a laboratory division.

A set of dissecting instruments (including fine pointed and heavy forceps, a strong pair of scissors, and at least two scalpels of different sizes) for each student.

A supply of water hones for sharpening scalpels.

A medium hard (2H or 4H) drawing pencil, eraser, and large note book ( $10 \times 14$  inches) of good smooth quality, but not too heavy paper, for each student.

A supply of small hand saws, chisels, wooden mallets, and bone forceps, and special knives, sufficient for each student or each two students in a laboratory division.

Some form of manifolding apparatus.

Small protractors for measuring the angles of bones.

Several long thin knives for sectioning brains.

Several hand section cutters.

At least one good microtome, and extra microtome knives.

At least one large (1000 c.c.) and one small (100 c.c.) glass graduate.

Small glass medical (male urethral) syringes for the injection of circulatory systems. (These, when connected by short pieces of rubber tubing with cannulas made by drawing out small glass tubing, are most satisfactory for injection of blood vessels.)

A paraffine oven or other device for imbedding (cf. p. 24).

An optical model of the eye (the simple one manufactured by the Harvard Apparatus Company, is recommended).

Some form of stethoscope.

Some form of sphygmograph.

Some form of sphygmomanometer.

Some form of hæmocytometer.

Some form of hæmoglobinometer.

An hydrometer (for fluids heavier than water).

A centrifuge machine (of the type used for examination of milk).

A circulation scheme for study of the mechanical principles of circulation (the one devised by Porter, and furnished by the Harvard Apparatus Company, has been found very satisfactory).

A respiration scheme for the study of the mechanical principles of respiration (devised by Porter and furnished by the Harvard Apparatus Company).

A pneumograph, writing tambour, and kymograph (Harvard Apparatus Company).

A spirometer.

Measuring tapes.

Large anthropological calipers (technically known as the pelvimeter).

An anthropometer.

Access to an incubator.

### VI. MISCELLANEOUS EQUIPMENT.

Large tubs or tanks with tight covers for the preservation of class material, of some material (e.g., glass, enamel, or papier maché) which will not be corroded by bichloride of mercury.

An ample supply of double crystallizing dishes, about 8–10 inches in diameter, and 3–4 inches deep, of heavy glass, one of which may be used as the cover for the other in the preservation of individual student material (such as brains), while either dish may be used as a temporary container for material which should be studied under water.

A supply of some form of modeling clay, e.g., plastilina.

An ample supply of small glass phials for the handling of histological material.

A supply of small glass tubing and rubber tubing to fit it.

Microscope slides, cover-slips, watch crystals, pipettes, and slide labels.

Absorbent cotton, filter paper, pure tissue paper for cleaning lenses, absorbent gauze for cleaning slides and cover-slips, an abundant supply of coarse unbleached cheese cloth for wrapping material for preservation, heavy manilla tags for labeling material.

Sets of reagent bottles, small dropping bottles, staining jars, balsam jars, and alcohol lamps.

General reagents: Formalin (40% formaldehyde); 95% alcohol (for all except histological purposes, denatured alcohol may be used if more convenient); corrosive sublimate in the form of large crystals (a tank or tub of saturated solution of corrosive sublimate

may be kept constantly ready for use by the simple device of suspending from the top a cheese cloth bag containing crystals of the sublimate). Absolute alcohol or acetone; xylol or turpentine; paraffine (the commercial "parawax" for household use proves of excellent quality for ordinary imbedding, and is inexpensive); albumen fixative; Canada balsam; iodine (in an aqueous solution of potassium iodide); methylene blue (aqueous solution); hæmatoxylin; eosin (in aqueous or alcoholic solution); Wright's stain for blood; glacial acetic acid; glycerine. For injection of blood vessels, pure gelatine (in thin sheets), corn starch, and various dry pigments in fine powdered form such as are used by house painters.

#### VII. FRESH MATERIAL.

One full-grown guinea pig (or other small mammal) for each student (or two students) for the general study of viscera.

One full-grown rabbit for each two (or four) students, to be hardened and preserved for the study of muscles. Certain of these may be used first for the demonstration of the general distribution of muscles, and then preserved for later dissection.

One pig's foot (anterior or posterior) for each student (or each two students).

One head of sheep or calf for each student (or for each two students).

One fresh mammalian eye for each student.

Several short lengths of spinal cord (lamb, calf, pig, or cat), preferably from different regions, hardened and preserved for macroscopic study, for each laboratory division of twelve students.

One set of thoracic viscera ("plucks") for each two students; these should have the trachea, larynx, and tongue attached, and at least one in each laboratory division should have the entire liver, and as much as possible of the diaphragm.

One cat, rabbit, or other mammal for each student (or each two students) for injection of blood vessels. These may be preserved and dissected later.

One or more intestinal tracts of cat for each laboratory division of twelve students, to be hardened and preserved and cut into short lengths for the macroscopic study of the alimentary canal. One simple kidney (rabbit, or guinea pig) for each student. These may be removed from specimens used for other purposes and preserved until needed.

One beef (veal), and one pig or sheep (lamb) kidney for each small group of students.

Several gravid females of various species of mammals, for demonstration of the uterus and embryo, with the relation of extraembryonal parts.

Occasional specimens of Necturus, small salamanders, frogs, rats, guinea pigs, rabbits, and cats for demonstrations, or for material for histological study; also occasional small supplies of fresh material for histological study from the market or abattoir.

#### VIII. BOOKS.

Among the numerous excellent, well-known text and reference books of human and comparative anatomy, histology, physiology, and embryology, with which every laboratory is presumably equipped, the following books may be mentioned as of particular value in connection with the course here outlined:

Bass and Johns, Laboratory Diagnosis. Rebman, New York. Beddard, Edkins, Hill, Macleod, and Pembrey, Practical Physiology. Arnold, London.

**Brubaker,** A Textbook of Human Physiology. Blakiston, Philadelphia.

Chauveau (English translation by Fleming), The Comparative Anatomy of the Domesticated Animals. Appleton, New York.

Dahlgren and Kepner, Principles of Animal Histology. Macmillan, New York.

**Davison,** Elements of Mammalian Anatomy, with especial reference to the Cat. Blakiston, Philadelphia.

Ellenberger and Günther, Histologie der Haussäugertiere. Parey, Berlin.

**Fiske,** An Elementary Study of the Brain, based on the dissection of the brain of the Sheep. Macmillan, New York.

Flower, Osteology of Mammalia, Macmillan, New York.

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